The Journal of Laboratory and Clinical Medicine

VOL. XVI ST. LOUIS, MO., OCTOBER, 1930 NO. 1

CLINICAL AND EXPERIMENTAL

THE EFFECT OF THYROPARATHYROIDECTOMY ON EXPERIMENTAL GASTRIC DEFECTS*

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IN 1926, it was suggested[1] as a result of some clinical and theoretical observations, that there is a disturbance present in patients with chronic gastric or duodenal ulcer, which consists in a generalized hyperirritability of the entire organism.

The precise nature of this hyperirritability, or rather the underlying cause, was not determined. Among other possibilities, because of the exaggerated reaction to mechanical, electrical, and pharmacologic stimuli, a latent tetany was considered as an explanation.

With the thought that perhaps such a general state may be an important factor in the pathogenesis of ulcer, we have attempted to induce such a condition in animals in order to study its effect on a defect experimentally instituted in the animal's stomach. It was hoped, in this way, to produce chronic lesions analogous to those in human beings. Drs. Neuhof and Ziegler, in a series of unpublished experiments performed in this laboratory on the rate of healing of ulcers excised in the stomach wall of dogs, found that lesions 5 cm. in diameter, extending down to the serosa, healed in fourteen to twenty-one days. Using their findings as a standard of comparison, our experiments were carried out as follows:

A total thyroparathyroidectomy was performed in dogs in the usual manner. The dogs were then placed on a carbohydrate and milk diet, and large doses of calcium were administered orally and intravenously. At the same time, or five days later, an ulcer was excised in the stomach on the lesser curvature at the incisura angularis. By the use of a thimble, from the

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Received for publication, April 5, 1930.

outside of the stomach, the lesser curvature could be everted through a small anterior gastrotomy wound, and a circular lesion 5 cm. in diameter could be easily excised extending down to the serosa (Fig. 1-A). These experiments were commenced in 1924, before the discovery of the parathyroid hormone by Collip, and these preliminary experiments were all unsuccessful. Twenty dogs died either within a few days in typical tetany convulsions, or lived ten to fifteen days, and then died in great asthenia and emaciation. Because of this, all of our observations on the defects instituted in these animals were discarded. The same procedure was carried out in four cats. One cat survived and remained in good health. On the forty-third day, the cat was sacrificed, and the ulcer found perfectly healed.

After the discovery of the parathyroid hormone in 1925,[2] the problem was again attacked. Thirty-three dogs were thyroparathyroidectomized, and then given Collip's parathyroid extract, in the form of Lilly's Parathormone, daily, or every other day. Two weeks later, a defect as described above, was excised in the stomach. After that, the parathormone was discontinued. Occasionally, a single dose of the hormone was necessary to control an isolated

A. *B.* *C.*

Fig. 1-A, Ulcer practically as excised (twenty-four hours), *B*, Healed ulcer, seventy-two days. *C*, Healed ulcer, forty-eight days.

convulsion. Of these 34 dogs, 24 died in tetany. In 9 dogs, the experiments were successful, and they are described below:

Experiment 8095. Collie, male: Extracapsular thyroparathyroidectomy. Twenty units of parathormone was given second day postoperative, then five units was given every other day for two weeks. Parathormone was stopped. Blood calcium two days later, 8 mg., three days later 7.3 mg. per 100 c.c. Ulcer was excised at the incisura angularis, twenty-eight days after the original operation. The course was uneventful thereafter. Forty-nine days later, the dog was chloroformed, and the interior of the stomach was inspected. Lesion was found completely healed.

Experiment 8102. Fox Terrier, male: Extracapsular thyroparathyroidectomy. Parathormone was administered as in previous experiment. Blood calcium forty-four days later, 9 mg. Ulcer was excised twenty-one days after first operation. This dog had occasional convulsions, and seemed inactive and depressed although there was no loss in appetite or weight. Dog was killed by etherization on the forty-seventh day, and stomach was inspected. Lesion was found completely healed.

Experiment 8112. Collie, female: Same procedure. Blood calcium 9 mg. and 8 mg. Ulcer excised thirteen days after first operation. Twenty-three days later, the animal had a severe tetany convulsion requiring twenty units of parathormone. Fifty-seven days after the ulcer excision, under ether anesthesia, the area of the stomach where the ulcer was excised, was resected and inspected. The ulcer was completely healed. Another similar ulcer was excised higher up. Ninety-two days later, again under ether anesthesia, the second ulcer area was inspected, and this ulcer was completely healed. Two more ulcers, one excised, and one made with the actual cautery, were instituted. Fifty-three days later, the dog was chloroformed, and both of these lesions were also healed. This dog had been under observation seven months, four ulcers excised, and lived without parathyroid extract except during the first two weeks.

Experiment 9450. Collie, female: Same procedure except that the ulcer was excised first, and the edges then seared with actual cautery. The dog had one convulsion after the parathormone was stopped. Thirty-eight days after the institution of the ulcer, the stomach was inspected, and the ulcer found completely healed.

Experiment 9014. Terrier, female: Same procedure. Seven days after the parathormone was stopped, the dog had a mild convulsion. Seventy-seven days after the ulcer was excised, the dog died in tetany. The ulcer was found completely healed. (See Fig. 1 *B*.)

Experiment 9053. Terrier, female: Same procedure. Fifty-seven days after the ulcer excision, the dog was chloroformed. The ulcer was found completely healed.

Experiment 9074. Collie, male: Same procedure. Thirty-four days after the ulcer excision, the stomach was inspected. Ulcer found completely healed.

Experiment 9495. Collie, male: Same procedure, except that the ulcer was first excised, and then the edges seared with actual cautery. The dog had one convulsion after the parathormone was stopped. Forty-eight days later, the ulcer was found completely healed. (See Fig. 1 *C*.)

Experiment 9507. Collie, female: Procedure same. The dog had an occasional tetany convulsion after the parathormone was stopped. Dog died in tetany ten days after the ulcer was excised, but the ulcer was found completely healed.

COMMENT

It is apparent from these experiments that, despite the thyroparathyroidectomy and the existence of the latent tetany, an ulcer placed in the favorite site for human ulcer, at the incisura angularis, will heal completely. In 49, 47, 57, 92, 53, 38, 77, 57, 48 and 10 days respectively, the ulcers were found perfectly healed. Altogether, 10 ulcers in animals in seemingly good health although in latent tetany, were observed to heal in these periods. It is possible that some of these defects took longer to heal than the fourteen to twenty-one days observed in the control experiments of Neuhof and Ziegler. However, in our experiments, the lesions were purposely not inspected ear-

lier because we felt that, unless the defect was unhealed for two or three months, it was not analogous to chronic ulcer in the human. The conclusion seems inevitable that this type of hyperirritability does not influence the rate of healing of gastric defects in the dog to any great extent. In criticism of these experiments, it may be pointed out that the thyroids as well as the parathyroids were removed. Also, that the degree of tetany could not be carefully controlled. Perhaps an animal which survives a parathyroidectomy is so well adjusted that latent tetany does not really exist. Against this, however, is the fact that five of the dogs had occasional tetany convulsions, and one died in tetany, yet the ulcer healed. Furthermore, the clinical studies which led to these experiments, although definitely evidencing hyperirritability, did not point conclusively to a parathyroid tetany. It is known that hyperirritability, often presenting the clinical features of tetany, can be brought about in other ways than by parathyroid insufficiency. It will suffice to mention here the tetany of pyloric obstruction (gastric tetany), alkalosis tetany, and guanidin tetany. Further experiments on the effect of these types of hyperirritability on gastric and duodenal mucosal defects should be carried out.

A review of the literature on tetany in its relation to the gastrointestinal tract will not be attempted here. This work was done, and is reported only because the clinical studies mentioned in the introduction seem to indicate that, possibly, a profitable method of approaching the ulcer problem would be to institute in the animal the general disturbance which appears to be present in human beings suffering from chronic gastric and duodenal ulcer, and then to observe the effect of such a general disturbance on a gastric or duodenal lesion.

SUMMARY

1. Patients with chronic gastric or duodenal ulcer present a hyperirritability somewhat suggestive of latent tetany.

2. To investigate a possible relationship, a thyroparathyroidectomy was performed in 60 dogs and 4 cats. Twenty dogs and 3 cats died in tetany without the administration of parathyroid extract. Twenty-four dogs died in tetany, who had received parathyroid extract for two weeks only.

3. Nine dogs, after two weeks of parathyroid extract administration, lived normally without it, and in these, an ulcer excised in the stomach at the incisura angularis, was found healed in 10, 38, 47, 49, 53, 57, 34, 48, 77 and 92 days respectively.

CONCLUSIONS

1. A circular gastric defect, 5 cm. in diameter, extending down to the serosa, in the lesser curvature at the incisura angularis heals completely in approximately one to two months in an animal in whom a thyroparathyroidectomy has previously been performed.

2. Apparently, neither a thyroid insufficiency nor a latent tetany interferes with the healing of a gastric lesion in the dog or cat.

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CREATINURIA IN TUBERCULOSIS*

I. Creatinuria in Tuberculous Men

By James T. Thompson, Denver, Colo.

INTRODUCTION

THIS work had its inception after marked creatinuria was observed in a single individual who had been critically ill from pulmonary tuberculosis. It was extended to include several other men in various stages of the disease. Nine of these men were selected at random by C. C. Davis, of Albuquerque, New Mexico, from among his patients. Four others and the controls were in the Colorado General Hospital at Denver.

An examination of the literature reveals that while creatinuria has been reported in many pathologic and experimental conditions, creatinuria in tuberculosis has attracted little if any attention heretofore. Certainly no particular significance (if indeed any exists) has been attached to it. Nor is it the purpose of this paper to attempt an interpretation of creatinuria in tuberculosis. Our sole intention is to report the presence of creatinuria as a frequent concomitant of pulmonary tuberculosis.

Additional analyses on a large number of individuals will be necessary to establish the exact status of creatinuria in this disease. Great variation in the ratio of urinary creatine to creatinine and in the creatinine coefficient exists. Some diseases show a more pronounced creatinuria than is observed in tuberculosis. In fact one "terminal" case of pulmonary tuberculosis had no creatine in the urine. Because of the varied manifestations of this condition, further investigation will be required before it is known whether any significance can be attached to it. Such work is in progress now, and indicates that the presence of urinary creatine may signify a physiologic disturbance of considerable importance in certain cases.

The scope of this article does not permit a review of the vast work done upon creatinuria, nor is such an attempt necessary. A thorough review of this literature is available in Hunter's (1928) recent monograph *Creatine and Creatinine.*

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EXPERIMENTAL

The urine was collected in clean bottles containing toluene and was analyzed by Folin's colorimetric method. No acetone bodies were present in any of the specimens; nor was there any medication which might influence the findings. The men were on normal diets. None of the cases for which figures are presented were considered terminal. The urine was usually distinctly acid to litmus.

The question of creatinine coefficients shall not be gone into as no significance can be attached to this factor at present. The coefficients ran all the way from below to above the reported figures for normal males. In Cases 9 and 12, the coefficient is extremely low, being 6.5 and 4.7 respectively.

Since creatinuria was found with such constancy in the urine of tuberculous men, the technic and chemicals used throughout were checked by running the tests on urine of normal men.

The results obtained from twenty-four-hour urine samples of thirteen tuberculous men are given in Table I. Two control cases are also included in this table.

With the exception of the first and last five specimens in Table I, which were studied in Denver as controls, all "a" specimens were collected on September 15, 1929, and run as soon thereafter as possible. These determinations were made in Albuquerque. All "b" specimens were collected in Albuquerque by Dr. Davis on November 8, 1929, and shipped to Denver where studies were begun on November 11, 1929.

Throughout this work, it has been the policy to obtain the preformed creatinine figure as soon as possible. This figure does not remain constant for a period of weeks as does that for the total creatinine. When possible the total creatinine determinations and those for preformed creatinine were done at the same time. But since it requires more time to run the former, a lapse of twenty-four hours would sometimes intervene between determinations when quantity studies were being made.

The specimens obtained in Denver were usually run immediately after they had been voided. To accomplish this, 4 six-hour specimens were usually obtained and the total twenty-four-hour figures derived by addition of the four separate determinations. Creatine was not found in every six-hour specimen, nor was it present in the urine voided at the same time of day by each individual. The time of creatine excretion seemed to be more or less constant for the individual, generally some time during the night.

Only in the four cases where duplicate readings are given ([a] in each instance for September 15, 1929, and [b] for November 8, 1929) does sufficient time elapse between determinations to make comparisons worth while. In these four cases, clinical findings as supplied by Dr. Davis are interesting.

Case 4 had been troubled with "colds" and "rheumatism" prior to being placed on rest about September 1, 1929. In November, Dr. Davis wrote, Case 4 "has had a relapse since you were here and is running 100° to 101° F. temperature with a complicated tuberculous fistula and tuberculous throat. You know he is that widespread diffuse pulmonary case that did not have any

TABLE I

CASE	AGE	SAMPLE	VOLUME IN C.C.	TOTAL CREATININE N IN GM.	PREFORMED CREATININE N IN GM.	CREATINE N IN GM.	TEMPERATURE	PER CENT OF CREATINE	REMARKS
1	32	a	1240	0.7487	0.6058	0.1429	97.6°	19.1	Old thoracoplasty. Working.
2	44	a	620	0.5623	0.4793	0.0830	normal	14.7	Old pneumothorax, arrested. Working.
3	24	a	1400	0.6400	0.5828	0.0572	normal	8.8	Incipient. Bed rest.
4	35	a	1300	0.8601	0.4884	0.3717	N–99.6°	43.1	Widespread diffuse pulmonary case.
		b	713	0.8219	0.7179	0.1040	100–101°	12.6	Complicated tuberculosis. Bed rest.
5	21	a	1510	0.8755	0.7464	0.1291	100–101°	14.7	Active, ''third stage case.'' Bed rest.
		b	766	0.7868	0.6547	0.1321	normal	16.8	Gain of 18 pounds.
6	23	a	510	0.4204	0.2995	0.1209	N–100°	28.7	Active, emaciated. Bed rest.
7	25	a	970	0.5768	0.4290	0.1478	normal	25.6	Pneumothorax. Bed rest.
		b	878	0.4350	0.3401	0.0949	normal	21.8	''Rapid improvement.'' Bed rest.
8	21	a	810	0.8162	0.6322	0.1840	N–99.4°	22.5	''a'' on bed rest; ''b'' light exercise.
		b	964	1.0090	0.9044	0.1046		10.4	Condition apparently unchanged.
9	36	a	2200	0.4906	0.4161	0.0745	N–100°	15.2	Long standing. Bed rest.
10	30	a	2610	0.9490	0.8325	0.1165	normal	12.3	Incipient. Bed rest.
11	24	a	1099	0.6745	0.6312	0.0433	normal	6.6	Taking pneumothorax. Working.
12	26	a	2816	0.3522	0.2671	0.0851	100–103°	24.1	Advanced pulmonary tuberculosis. Left spontaneous pneumothorax.
13	48	a	2702	0.5017	0.4442	0.0575	100–102°	11.4	Unilateral cavitation in left lung, right apex involved.
14	25	a	1642	0.5194	0.5194	none	normal	0	Bed rest. Appendicitis, chronic. No complications.
15	49	a	1295	0.5176	0.5176	none	normal	0	Some exercise. Gastric ulcer, lungs negative.

breaking down in his chest at the time you were here." His creatine output had, in less than two months' time, decreased from 43+ to 12+ per cent of the total creatinine.

Of Case 5, Dr. Davis wrote, he "is a third stage case but I was unable to do anything specific for him except put him on bed rest. As you remember at the time you were here in September he was running 100° to 101° F. temperature; was coughing and raising four ounces in twenty-four hours. At the present time his sputum has reduced to one-half ounce in twenty-four hours; his temperature is normal and he has gained eighteen pounds in weight." This change occurred within a period of seven weeks. With this improvement there was an insignificant increase in creatine output, but a noticeable decrease in total creatinine. This latter fact is quite surprising when it is coupled with 18 pounds gain in weight.

Case 7 "is improving very rapidly —. He has no fever, cough or sputum." An appreciable decrease in his total creatinine excretion is noteworthy.

Case 8 was apparently overnourished in September. In November, Dr. Davis wrote that he "is still running a low-grade temperature and so far as I can see his condition is unchanged. I have him on thirty minutes exercise and have carefully checked his chest every two weeks but this shows no evidence of reactivation." With his decrease in creatine and great increase in total creatinine excretion, he may present some interesting results at a later date.

Several unavoidable factors have made it necessary to make careful control studies to validate the findings presented in Table I. There are at least five factors which might possibly affect the urine in a manner sufficient to negate this entire work. Table II contains a summary of control studies which are intended to demonstrate the effect of these five factors which are:

1. Effect of standing at 78° F. upon creatine-creatinine content of urine.
2. Effect of standing at 38° F. upon creatine-creatinine content of urine.
3. Effect of shipping upon the creatine-creatinine ratio in urine.
4. Influence of bed rest on creatine content of urine.
5. Influence of diet on creatine content of urine.

Since none of these factors affected a change in the total creatinine determinations, this figure is given only once for each urine studied. The preformed creatinine and creatine figures are given in each instance. All determinations are for nitrogen and are written in grams. Fractional determinations are given for the twenty-four-hour urines of Cases 11 and 13.

Pertinent remarks concerning all the control cases except Cases 16 and 17 are included in Table I. Both these cases present negative lung findings. Case 16 has "toxic arthritis or low-grade infectious arthritis of the feet and back." Case 17 was provisionally diagnosed as having Buerger's disease. None of these men were at an age when creatinuria might be considered a normal urinary excretion.

The patient, Case 1, was placed on a creatine-free diet for a time. Creatinuria was not completely eliminated, although there was a slight reduction in both the creatine and creatinine content of the urine during this period.

TABLE II

CASE NO.	VOLUME IN C.C.	SPECIFIC GRAVITY	REACTION TO LITMUS	AGE OF PATIENT	FRESH URINE			STANDING AT 78° F. 4 TO 6 DAYS		AFTER SHIPPING 4 TO 5 DAYS		REFRIGERATOR 8 TO 10 DAYS		SHIPPED OR STANDING URINE RETURNED TO REFRIGERATOR FOR 2 DAYS	
					TOTAL CREATININE	PREFORMED CREATININE	CREATINE	PREFORMED CREATININE	CREATINE	PREFORMED CREATININE	CREATINE	PREFORMED CREATININE	CREATINE	PREFORMED CREATININE	CREATINE
14	1642	1.014	Neut.	25	0.5194	0.5194	0.0000	0.4694	0.0500	0.4422	0.0772	0.5194	0.0000	0.5043	0.0151
15	1295	1.02	Acid	49	0.5176	0.5176	0.0000	0.5176	0.0000	0.5176	0.0000	0.5176	0.0000	0.5176	0.0000
16	1434	1.014	Neut.	38	0.5552	0.4426	0.1126	0.5125	0.0427	0.5125	0.0427	0.4421	0.1126	0.5125	0.0427
17	451	1.023	Acid	23	0.4440	0.4039	0.0401	0.3898	0.0542	0.3898	0.0542	0.3898	0.0542	0.3898	0.0542
11	196	1.029	Basic	24	0.1375	0.1375	0.0000	0.1375	0.0000			0.1375	0.0000	0.1375	0.0000
	903	1.022	Acid		0.5370	0.4937	0.0433	0.4859	0.0433			0.4937	0.0433	0.4937	0.0433
12	2816	1.012	Acid	26	0.3522	0.2671	0.0851	0.2671	0.0851			0.2671	0.0851	0.2671	0.0851
13	468	1.006	Acid	48	0.0686	0.0686	0.0000	0.0609	0.0077			0.0686	0.0000	0.0609	0.0077
	1761	1.007	Acid		0.2569	0.2128	0.0441	0.1805	0.0764			0.2128	0.0441	0.2128	0.0441
	473	1.013	Acid		0.1762	0.1628	0.0134	0.1628	0.0134			0.1628	0.0134	0.1628	0.0134

The observed effect may be due to fluctuation which can be the result of factors other than diet and which cannot be discussed here. The study will be continued in Case 1 and eventually extended to include other individuals. In this manner it is hoped to determine whether the creatinuria of tuberculosis is due to the organism's inability to retain exogenous creatine, or to its inability to properly metabolize the endogenous product. Both possibilities may be involved.

Some experimental results obtained from apparently normal adult male guinea pigs inoculated with virulent tubercle bacilli show that as much as 65 per cent of the creatine-creatinine total may be in the form of creatine. These animals were maintained on an adequate creatine-free ration. Consequently exogenous creatine cannot be a factor in this guinea pig creatinuria.

The partial destruction of certain organs is revealed at autopsy of these guinea pigs. This destruction may account for the creatinuria. The particular organ involved, if it is a single organ, is unknown at this time. Experiments along this line are being continued in the hope of determining, if possible, the extent of tissue destruction necessary to result in creatinuria.

DISCUSSION

Results in Table II are necessary for validation of Table I. The latter indicates that creatinuria is present in every case of pulmonary tuberculosis presented. But since facilities in Albuquerque were not ideal for the collection and storage of urine specimens prior to analysis, it has seemed necessary to study the effect of standing at 78° on a number of controls. It was also considered desirable to study the urine of tuberculous men under ideal circumstances.

Creatinine may be converted to creatine upon standing; this reaction is reversible. But in none of my controls, which stood several days, was the change sufficient to account for the creatinuria observed in the urine analyzed in Albuquerque. Furthermore these Albuquerque determinations were delayed for a period of only twelve hours at the most. Many urines show absolutely no change in a greater length of time. Then, too, the urines of Cases 1, 11, 12, and 13 were analyzed in Denver immediately after voiding and the determinations confirm the Albuquerque results. While a small portion of the creatine in the Albuquerque urines may be the result of a few hours delay in analysis, this factor cannot possibly account for the greater portion of this substance present.

There is a question as to the effect of shipping in Samples 4b, 5b, 7b, and 8b. These urines were shipped during cold weather and were three days in transit. They were placed in the refrigerator immediately upon receipt. Some specimens remained at refrigerator temperature for three days before it was possible to make a preformed creatinine determination.

Control specimens were shipped to Albuquerque and returned in April, 1930. The weather was warm and the round trip required five days. Results given in Table II show that shipping has the same negligible effect as standing at 78° F. for an equal period of time. Furthermore, results from placing control urines in the refrigerator for two days after shipping or standing

indicate a marked tendency for the urines to return to their original constitution. Cases 13 and 14 illustrate this phenomenon. This fact suggests that there is an apparent creatinuria sometimes produced by standing at 78° F., but that the actual condition evinces itself after the urine has stood for a time at refrigerator temperature.

Another fact, which indicates that the creatine in these shipped specimens is not an artefact, is that each of these twenty-four-hour urines was composed of from 3 to 4 fractional specimens, some of which contained no trace of creatine.

Lastly, there is the question of diet and bed rest. Cases 1, 2, and 11 were on regular diets and were working. Still they show creatinuria. Cases 14 and 15 were normal men on bed rest and hospital diet containing plenty of milk. The urines of these men contained no creatine. Additional examples of such controls would be desirable, but there is a certain amount of difficulty in finding normal young men on constant bed rest.

SUMMARY

These findings clearly indicate the presence of creatinuria in pulmonary tuberculosis, febrile or otherwise. It is realized that many more cases must be examined to prove its invariable existence in this disease.

The repeated analyses given for four individuals of the group demonstrate not only a fluctuation in the creatine output over a period of time, but a variation in the total creatinine production as well. Whether or not these changes in the amount of creatine and creatinine excreted are of any prognostic value cannot be told at this time from such limited observations.

Urine specimens may stand at refrigerator temperature for days without any change in either the creatinine or creatine content. Urine may stand at room temperature or be in transit at a temperature of 78° F. for a period of several days without appreciably altering the creatinine-creatine ratio. Slight changes which may occur are corrected by permitting such urine to chill for two days before analysis.

Creatinuria may not exist in all cases or in all stages of the disease, but our observations lead us to believe that its omission is rare. The cause of this urinary abnormality is undetermined, but since it persists after the disease is arrested, it may be due to and a measure of certain tissue destruction during the active phase. The phenomenon of creatinuria in tuberculosis is not confined to man.

CONCLUSION

Creatinuria is often associated with pulmonary tuberculosis.

Indebtedness to Dr. C. C. Davis of Albuquerque, New Mexico, for his hearty cooperation; to Dr. Ivan E. Wallin of this department; Dr. Richard W. Whitehead of the department of Physiology and Pharmacology; and to Dr. Gerald B. Webb of Colorado Springs, Colorado, for their constructive criticism; and to Miss Mary Strickland of the Biochemistry department for many favors extended to me during the course of this investigation is hereby acknowledged.

CHRONIC STROPHANTHIN POISONING*

Unusual Disturbances of Cardiac Rhythm Due to Massive Overdosage

By Albert S. Hyman, M.D., and Harry J. Lowen, M.D., New York City

STROPHANTHIN poisoning is less frequently encountered in the cardiac clinics of this country than in those abroad. Strophanthus and its several pharmacologic preparations enjoy considerable vogue in France and Italy; it is also employed to great extent in Germany and Poland. In this country and in England the drug has been confined almost exclusively to those cases of circulatory failure which do not respond to digitalis. Occasionally, Strophanthus has been used to augment the action of digitalis after the latter has been given over long periods of time. The occurrence of strophanthin overdosage is thus of such relatively rare incidence that the discovery of such cases is always interesting.

During the past five years only three cases of Strophanthus overdosage have been seen in the cardiac clinics of the Beth David Hospital Dispensary; two of them were Italians and one German. The first two were patients who had been treated by a physician who had given them an Italian preparation containing about 1.5 c.c. of the tincture per dose; from 3 to 5 doses were being taken a day. Persistent and obstinate diarrhea with nausea and palpitation were striking symptoms in these two cases. Neumann[1] has reported two such cases of acute Strophanthus poisoning. Both showed diarrhea as the most prominent feature of the condition. Others[2] have also reported the gastrointestinal reactions to this drug when taken by mouth in large doses. It is also interesting to note that even when given by rectum Strophanthus preparations may produce the same clinical picture. Fuehner[3] has recently described a fatal case of strophanthin poisoning in which the drug was administered in special suppositories.

The gastrointestinal reactions displayed by patients who have been using Strophanthus led Cornwall[4] to administer the drug sublingually, and in a series of papers he pointed out that the rapid absorption by this method prevented many of the disagreeable by-effects noted in the other methods of employing this drug. Subsequently, however, Eggleston and White,[5] in an extensive survey of the problem, demonstrated that the absorption of strophanthin by the sublingual route was practically negligible.

Two of the three cases seen by us at the Cardiac Clinic are related in detail in order to demonstrate the unusual types of arrhythmia which developed from strophanthin overdosage.

*From the Cardiac Clinics of the Witkin Foundation for the Study and Prevention of Heart Disease, Beth David Hospital, New York.
Received for publication, April 14, 1930.

CASE 1.—M. J., storekeeper, aged fifty-two, arrived in New York from Italy about five years ago. He was apparently well up until the time he landed in this country, when he began to complain of shortness of breath and palpitation on effort. His work made it necessary for him to be constantly running up and down stairs with packages of considerable weight. It was not long before he found himself unable to continue his occupation, and he was taken to a physician who was practicing in the Italian community.

He was given a bottle of medicine and told to take a teaspoonful every three or four hours; this he did for about a week but on feeling no better he discontinued its use. He received no further medical attention for about a year; after a slight attack of pharyngitis he noted marked difficulty in breathing. These symptoms appeared to him so similar to those experienced previously that he decided to take the medicine again. Having finished

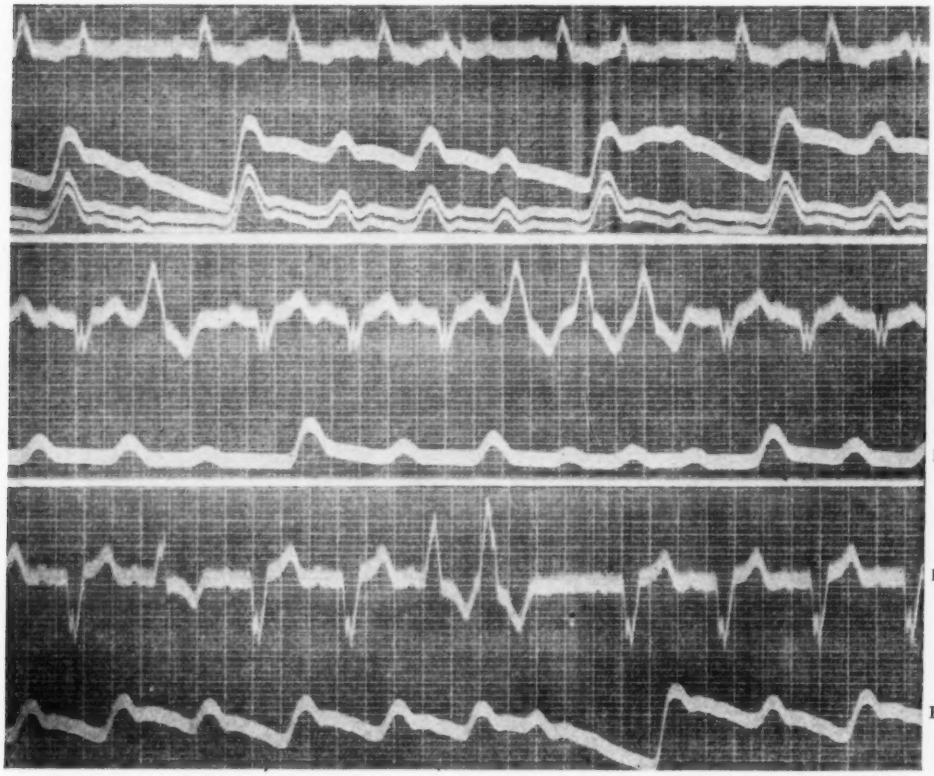

Fig. 1.—Simultaneous electrocardiographic and polygraphic tracings taken at the height of strophanthin overdosage. In this record and in the subsequent ones, the single line represents the pulsation from the radial artery and the double line from the tibial artery. The irregularity in the pulse is seen to be due to extrasystoles occurring from several foci; sometimes one focus may send out a series of stimuli as in Lead II. When this occurs, no pulse is felt at the wrist, but the next beat is so large that the patient experiences it as a definite thump (see Lead III). Note that there are T-wave alterations in the first lead and that the QRS complexes assume the configuration of bundle-branch block.

the bottle with some apparent relief, he sent a messenger to obtain more of the same preparation. This was given to him and he continued to take it for about a week. Noting no further improvement in his condition he decided to double its dose and took a teaspoonful every two hours. This he had been doing for three days prior to his first visit to us; when seen at the Cardiac Clinic he was complaining of palpitation, substernal distress, and more particularly nausea and a severe diarrhea.

A complete cardiovascular survey made at that time revealed a rather large heart with a marked aortic configuration. Blood pressure readings were systolic 155 and diastolic 95.

The heart sounds were of rather poor quality and a rough blowing systolic bruit was heard at the upper end of the sternum. The dominant rhythm of the heart appeared at times grossly irregular, and from a clinical point of view, it was impossible to distinguish whether the arrhythmia was due to auricular fibrillation or to many extrasystoles.

Electrocardiographic and polygraphic studies revealed the interesting condition seen in Fig. 1. The dominant rhythm of the heart was interrupted by extrasystoles occurring from more than one focus. At times a series of right ventricular extrasystoles would occur together and a short interval of paroxysmal ventricular tachycardia would occur. The rate in these attacks was so rapid that incomplete filling of the left ventricle occurred and as a

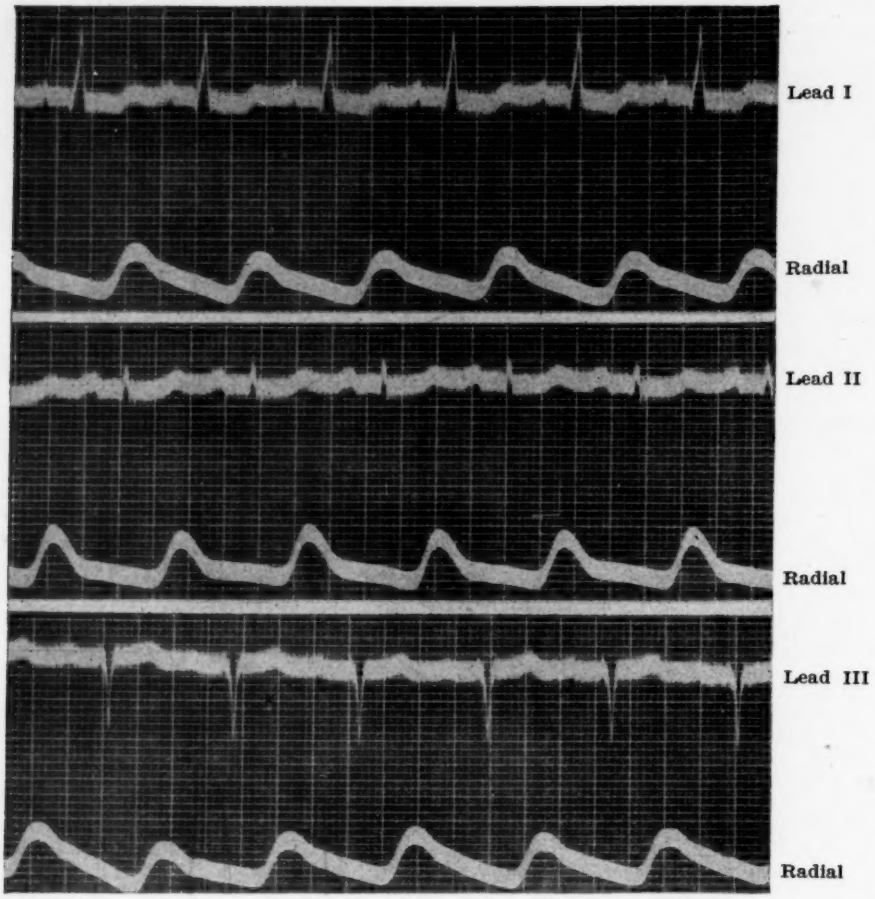

Fig. 2.—Records taken ten days after withdrawal of strophanthin. Note the entire disappearance of the extrasystolic arrhythmia with its associated hemodynamic disturbances. The configuration of the right bundle-branch block is practically gone except for a slight slurring in the initial limb of the complex. The T-waves are still altered in the first lead and the P-R interval remains delayed to 0.22 second.

result of which the aortic valves would fail to open. This pulse deficit could be felt at the wrist as a long pause, and the return of normal sinus rhythm was accompanied by such a large volume of blood expelled from the left ventricle that the patient experienced this sensation as a definite thump of the heart.

There was no edema of the legs but the liver edge was definitely palpable. The lungs were quite free from congestive failure. The patient was taken off the strophanthin mixture and given relatively large doses of theobromine sodium salicylate. He was seen again about ten days later only after urgent request. He felt so much better after the first few days that he had discontinued his treatment entirely. Electrocardiographic tracings (Fig. 2)

taken at this time showed no further evidences of the extrasystolic arrhythmia seen previously. There was still suggestive alterations of the T-waves in Lead I, the QRS complexes had returned to a transmission time of 0.10 seconds. This patient was seen again about six months later and he reported no further attacks of palpitation.

The mixture taken by this patient was subsequently shown to contain about $^{3}/_{100}$ of a grain of strophanthin per teaspoonful. On the three days in which he took a teaspoonful every two hours he received about $^{18}/_{100}$ grains of this drug. This is about four times that used in the experiments of Eggleston and White and five times that suggested by Kieth,[6] and Haag and Hatcher.[7] Levy and Cullen[8] have shown that aqueous preparations of strophanthin rapidly lose their potency and become inert in the presence of certain acid substances. The preparation used by the patient, however, was not more than a few days old, and it can be assumed that the strophanthin was still active. The extrasystolic arrhythmia which promptly disappeared on withdrawal of the drug suggests that the myocardium was made especially toxic and hyperirritable by this substance.

While the literature upon digitalis is especially voluminous, that in regard to strophanthin is rather limited. For this reason the specific pharmacologic action of strophanthin so far as it relates to the heart is not so clearly understood as that of digitalis. Apparently the two drugs affect the myocardium somewhat in the same manner; this is especially true in regard to the establishment of ectopic rhythms. We have previously shown[9] that digitalis overdosage is associated with the development of extrasystoles, first from a single focus and then by ectopic beats that arise from two or more foci. As overdosage increases, the number of extrasystoles may become very frequent, finally developing into a series which suggests a paroxysmal type of ventricular tachycardia. The step from such runs of extrasystoles to ventricular fibrillation may be a short one not without some hazard to the life of the patient.

Cornwall[10] has recently pointed out, however, that there are certain well-marked differences between digitalis and Strophanthus, in that the former affects contractility and tonicity through the vagal mechanism of the heart while the latter does not, furthermore Strophanthus increases myocardial irritability more than digitalis. This would suggest that the development of an extrasystolic arrhythmia would occur sooner during strophanthin administration than with digitalis. Case 2 is thus of considerable interest in demonstrating how such an extrasystolic arrhythmia may be so continuous as to be easily mistaken clinically for auricular fibrillation. Probably no differential diagnosis is more difficult to make; at the same time a correct understanding of the process is a matter of some concern so far as the treatment of the patient is concerned. Whereas digitalis would be the drug *par excellence* for the treatment of auricular fibrillation, the use of this drug when the arrhythmia is due to extrasystoles arising as the result of strophanthin overdosage may jeopardize the life of the patient.

CASE 2.—Woman, aged fifty-four. About ten years previously she began to complain of dyspnea and palpitation on moderate effort. She was treated by several physicians from time to time up until about four and one-half years ago, when she began to use strophanthin.

This product was prepared in France and contained 1/10 of a milligram of crystallized strophanthin (Ouabaine) in small granules. At first she said that she had taken two of these granules three times a day (0.6 mg.) with a considerable relief from her symptoms. She continued to take this dosage for about four months and then noting the return of her previous complaints she increased the dose herself to three granules three times a day (0.9 mg.). Some weeks later with the onset of edema of the legs she returned to the physician who had originally prescribed this medication and was somewhat surprised to learn that he had died.

Nothing daunted, she continued to take 0.9 mg. of digitalis daily for some months, gradually increasing the number of granules taken to combat certain symptoms which she believed were due to insufficient amounts of the drug. For the past two and one-half years she had been taking from 10 to 20 of these granules each day with the exception of Sunday. This she could measure more or less accurately due to the fact that the trade package containing this specific brand of the drug contained 60 granules; she had been in the habit of using from 1 to 2 of these packages in six days. This dosage had been maintained up until about one month ago when following a short attack of grippe she became exceedingly heart conscious and said that the palpitation became so great that she could not sleep. She then increased the dosage of strophanthin to about 2.5 mg. per day. When seen on the twenty-sixth day following the introduction of this new dosage, she was taking nearly 3 mg. a day.

Physical examination revealed rather a heavy obese woman, dyspneic, cyanotic, and complaining of great fatigue. The heart was considerably enlarged and the sounds were of very poor quality. The dominant rhythm was grossly irregular, averaging about 100 beats per minute. Her blood pressure was systolic 95 and diastolic 60. The liver was slightly palpable and tender; there was a marked pretibial edema and many moist râles were heard in both lungs. She was first seen by one of the internes of the Hospital who made a diagnosis of auricular fibrillation; she was admitted to the cardiac service and placed upon digitalis therapy. The usual dose of 2 grains of digitalis was ordered, but before the patient could take it, she was seized with terrific abdominal pain and began to vomit.

A few hours later electrocardiographic studies were made (Fig. 3). It was discovered that the gross irregularity which had been diagnosed as auricular fibrillation was, in fact, an extrasystolic arrhythmia with ectopic beats arising from multiple foci; the tracings showed as many as eight ectopic points of extrasystolic origin arising within the space of a minute. A more careful history then revealed that the patient was unquestionably suffering from a strophanthin overdosage. A sample of the granules that she had been taking was given to the Hospital Pharmacist for analysis; complete identification of the substance was subsequently confirmed from the trade packages which the patient had.

Complete withdrawal of the drug was promptly instituted and dextrose and metaphylline were administered intravenously. Within twenty-four hours the pulse rate had become more regular so far as individual beats were concerned, but it had developed a triphasic rhythm. This extremely unusual arrhythmia originally described by Kaufmann was noted clinically by examining the pulse rate from time to time. The dominant rhythm appeared to be at the rate of about 56 beats per minute; this rate would abruptly change to a rate of about 96 and then it would either return to the previous rate or drop to that of 30. These three rates would sometimes run regularly for a minute or more, first one and then the other.

Electrocardiographic and polygraphic studies made at this time revealed an interesting condition (Fig. 4). In these studies simultaneous polygraphic tracings were taken of the radial and tibial arteries with each of the three leads of the electrocardiogram. It was seen that the rate of 96 represented the normal sinus rhythm of the patient; when the rate dropped to 56, this was due to ineffective extrasystoles occurring after each normal cardiac cycle. Finally, the drop to the 30 beat rhythm was the result of alternating right and left ineffective extrasystoles. Fig. 4 is unusual in that all three rhythms can be seen in one tracing.

The change from one rhythm to another was described by the patient as being accompanied by a sense of giddiness. She was more comfortable at the 56 rate rhythm than

Fig. 3.—Lead III. Electrocardiographic studies taken on admission to the hospital directly following a paroxysm of painful heart consciousness. The tracing shows that the gross irregularity is not due to auricular fibrillation but to an extrasystolic arrhythmia which arises from many ectopic foci. The normal cardiac cycle (PRT) occurs irregularly; extrasystoles of many different configurations are seen at points *1*, *2*, *3*, *4*, and *5*. The interval from *1* to *4* represents but four seconds of time in which four different extrasystolic configurations are found.

Fig. 4.—Lead II. Electrocardiographic and polygraphic tracings of the radial and tibial arteries taken simultaneously during the transition period of triphasic arrhythmia. Note that the normal cardiac cycle (PRT) occurs more or less irregularly; when there is a series of normal cardiac cycles the rate is 96 beats per minute and is seen in the *1-2* interval above. When alternating types of ventricular extrasystoles arise, there is a complete blocking of the normal cycle for the space of three beats: this irregularity may occur many times in succession producing a radial pulsation of 30 beats per minute. Such a beat is seen in the *2-3* interval above. Finally, extrasystoles may occur after each normal cardiac cycle, and the subsequent refractory period reduces the pulse rate to about 56 beats per minute; this latter rhythm is seen in the *3-4-5* interval. While only a small number of beats in each rhythm can be shown in the record, each of these three rhythms might run for several moments rather regularly so that the impression of three distinct regular pulse rates would be given. It was for this reason that the term triphasic arrhythmia was given to the condition by Kaufmann.

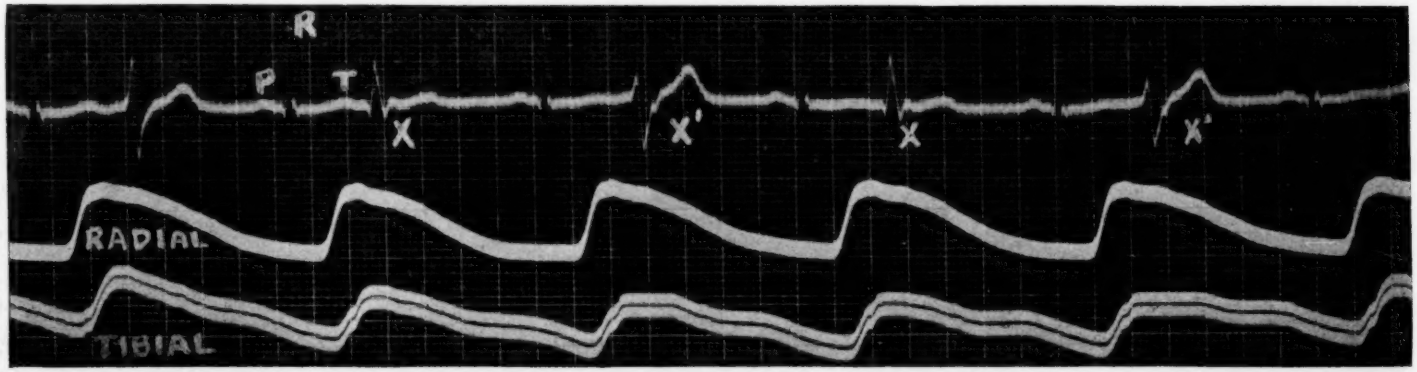

Fig. 5.—Lead I. Graphic records taken forty-eight hours after the withdrawal of strophanthin and after the intravenous administration of dextrose and metaphylline. Note that the pulse rates are now regular at 44 beats per minute. Each normal cardiac cycle is followed by an extrasystole; the extrasystoles now arise from only two ectopic foci, each one operative alternately. For example, in the record of the first cardiac cycle is followed by an extrasystole arising at focus *X*, whereas the next normal cardiac cycle is followed by one arising from focus X'. The next cycle is followed by an extrasystole arising at focus *X* and the following again from *X'* and so on.

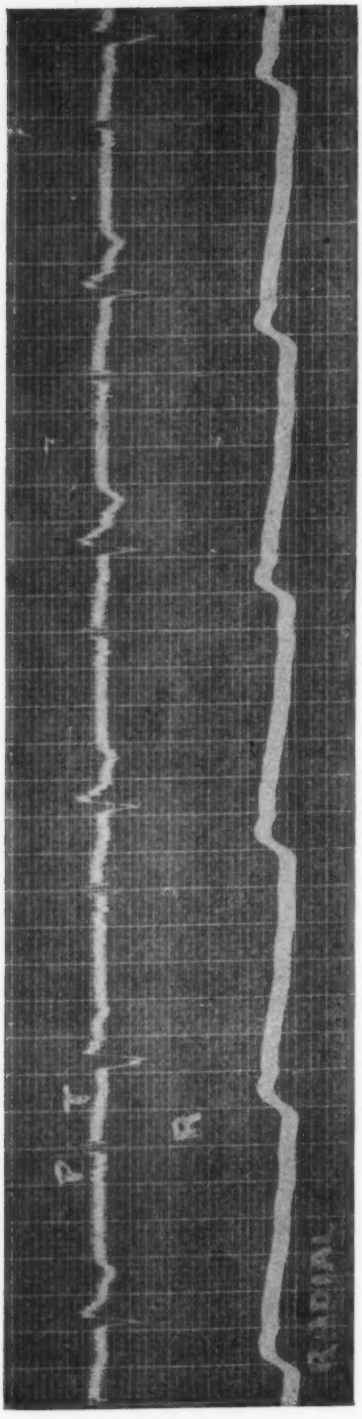

Fig. 6.—Lead III. Graphic records made the fifth day after admission. The pulse rate still remains regular at 44 beats per minute due to the blocking phenomenon of the extrasystolic arrhythmia described in the previous records. At this time, however, the extrasystoles are arising from a single focus.

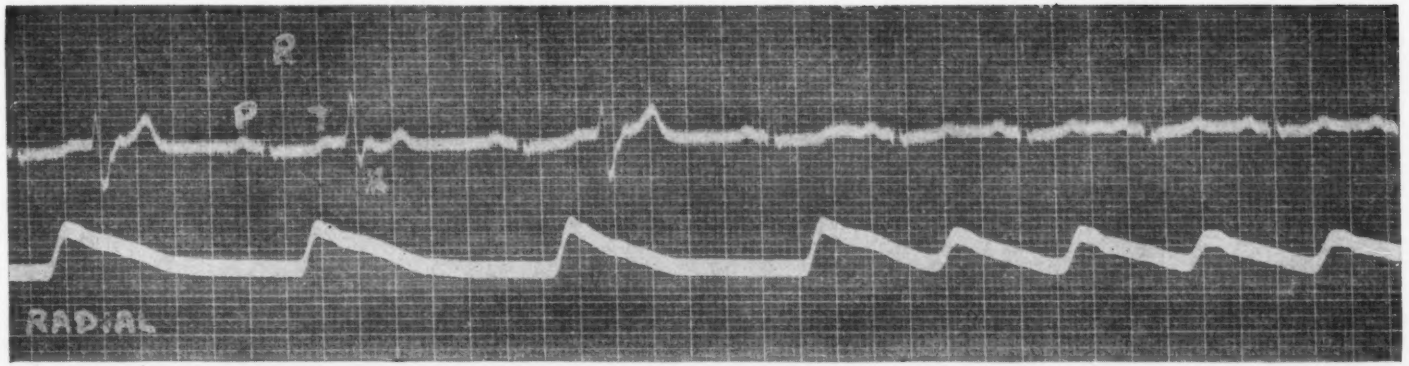

Fig. 7.—Lead I. Graphic records made on the following day showing the return to a diphasic rhythm. The extrasystoles which have blocked the normal cardiac cycle are now disappearing for more or less long intervals so that there are two regular pulse rates, one at 44 and the other about 84. In this record the transition between the slower and faster rate is graphically demonstrated.

the other two, although the 30 rate rhythm had with it the sensation of substernal distress. On the following day the rhythm was constantly maintained at a rate of 44 beats per minute. Electrocardiographic and polygraphic studies (Fig. 5) now showed an extrasystole following each normal cardiac cycle. These extrasystoles appeared to come from two different foci, first one and then the other in a regular order. No special treatment other than catharsis and sedatives were given to the patient who at this time had no special complaints except that of general weakness.

Three days later further electrocardiographic and polygraphic studies were made (Fig. 6). The rate still remained at 44 beats per minute, but this was due to ineffective ex-

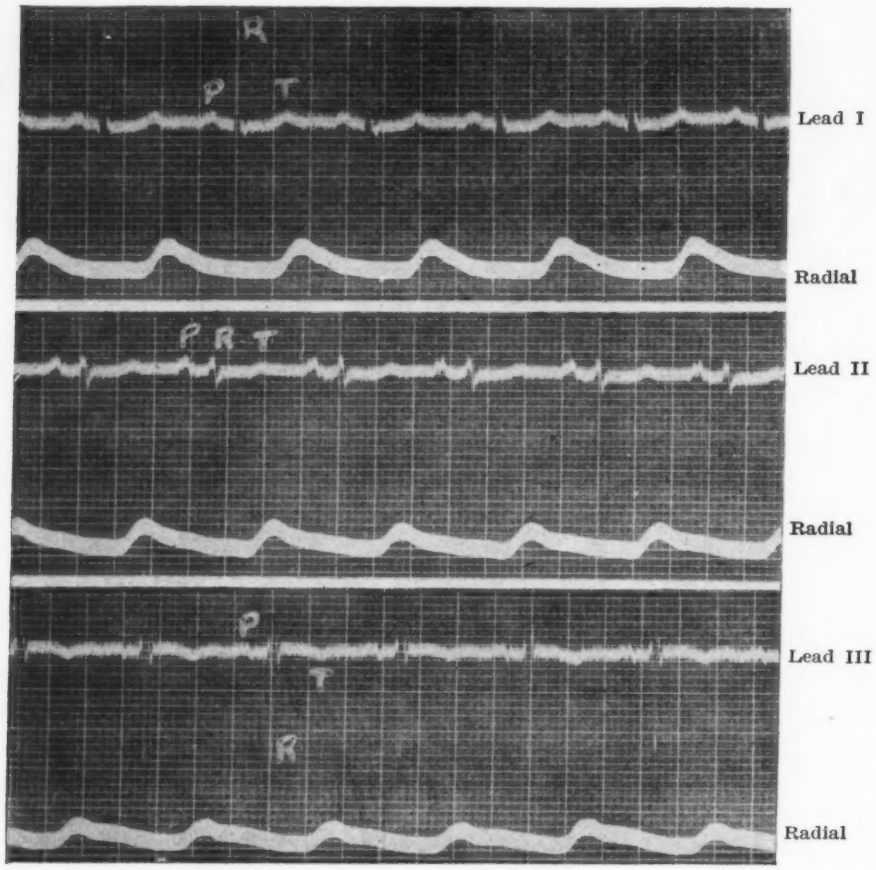

Fig. 8.—Graphic records taken on the tenth day after withdrawal of strophanthin. The normal cardiac cycle had returned, and the pulse rate averaged 86 beats per minute; the extrasystoles had disappeared except for an occasional one which occurred so infrequently that no record of it could be obtained.

trasystoles occurring after each normal beat; the extrasystoles at this time were now coming from a single foci.

The following day the pulse rate began to show a diphasic rhythm; periods of a regular pulse with a rate of 84 beats per minute alternated with intervals in which the rate was still 44. These two rates would run from five to ten minutes at a time without change. Fig. 7 shows graphic records taken at the time when there was a change from one rhythm to another. This alternation of the two rhythms persisted for about three days when the pulse became regular at the higher rate. Graphic records taken at that time (Fig. 8) show a normal sinus rhythm with a rate of 86 beats per minute. Except for an occasional extrasystole which developed, the patient had no other changes of rhythm, and two weeks after

her admission to the hospital she was discharged relatively free from symptoms. Her blood pressure rose to 144/86. The heart sounds were of better quality and a rough blowing systolic bruit could be heard at the upper end of the sternum. The edema of the legs had disappeared but the liver edge was still palpable. X-ray examination of the heart showed it to be still considerably enlarged. The patient was seen from time to time for the next six months and except for an occasional attack of palpitation and substernal distress she was relatively free of subjective complaints.

COMMENT

A review of this case reveals several extremely interesting considerations. The first is that of the huge doses of strophanthin which the patient had been taking. Starting with the equivalent of about $\frac{1}{100}$ of a grain per day she increased this dosage to about $\frac{1}{60}$ of a grain. This amount in turn was increased from time to time until for two and one-half years she was taking as much as $\frac{1}{30}$ of a grain a day. For a period of three weeks before admission to the hospital she had been taking about $\frac{1}{20}$ of a grain daily. Eggleston and White[5] in their study reported that when patients received daily doses of from $\frac{1}{90}$ to $\frac{1}{50}$ of a grain of strophanthin considerable distress was experienced; none of their cases continued such dosage for longer than four days. This dosage, however, was exceeded by that reported by Tracey[11] who gave as much as $\frac{1}{60}$ of a grain three or four times a day over a period of four months; both Eggleston and White in a critical survey of this latter statement have stated that if given in this large dosage the drug could not have been absorbed without hazardous results to the patient.

In the case reported here the dosage exceeds that recorded in the available literature for the period of time over which it was self-administered. An approximate calculation shows that the patient had taken more than 300 grains of strophanthin over a period of four and one-half years. The tolerance developed for the drug is shown by the fact that increasing doses were required to produce a satisfactory symptomatic effect so far as a patient was concerned. The absence of its accumulative effect is also strikingly revealed by the massive doses used during the latter part of the self-administration of the drug as well as the rapid disappearance of the symptoms of overdosage when the drug was withdrawn.

The second point of interest is the cardiovascular response to strophanthin overdosage. Accompanied by myocardial hyperirritability the electrodynamic phenomenon of the heart became so disturbed that many ectopic foci became operative simultaneously. Extrasystoles from many different foci so altered the normal cardiac cycle that the resulting arrhythmia was indistinguishable clinically from the gross irregularity of auricular fibrillation. While digitalis is the drug of choice in this latter condition, it must be strictly contraindicated following the intensive use of strophanthin, and if given, would have unquestionably done much to have jeopardized the patient's life. Without instrumental assistance, however, the correct diagnosis could not have been made.

The graphic records were extremely useful in explaining the unusual types of arrhythmia which developed subsequent to withdrawal of the strophanthin and the supportive general measures of therapy which were given.

The decrease in the number of ectopic points from which the extrasystoles arose was associated with the phenomena of three-phasic and two-phasic rhythms. This was particularly noticeable on the day following the patient's admission to the hospital when the pulse ceased being irregular; instead, the pulse rate assumed three separate regular rates one alternating with the other.

Diphasic alternation of regular cardiac rhythm is not infrequently seen in heart-block of the second degree or partial type and a rate which changes abruptly into ratios of 2:1 or 3:2 can be suspected clinically as being due to this condition. Extrasystoles, especially when they follow the bigeminal pattern, may also result in two rhythms one of which is twice as fast as the other. The alterations of such rhythms may occur two or three times a minute but in some cases one rhythm may persist for days or even weeks before the change occurs.

Triphasic rhythm, on the other hand, is not especially common and is usually due to extrasystolic phenomena; we have never seen this latter condition develop during heart-block. Triphasic rhythm is due to ectopic beats arising from multiple foci; generally, however, two foci predominate above the others. Such foci may occur in adjacent parts of the myocardium but more frequently they arise at points located in the two ventricles. When this occurs, the phenomenon of alternating right and left ventricular extrasystoles may be produced so that normal cardiac cycles are depressed. This results in three rhythms having a multiple of 3 as their functional ratio to each other; that is to say, the rates might be 90, 60, and 30 beats per minute. In Fig. 4 the rates are 96, 56 and 30 which more or less closely adheres to the theoretical calculation for this hemodynamic phenomenon. When, however, a diphasic rhythm occurs the two rates are usually multiples of 2; that is, one rate might be 80 and the other 40. In Fig. 7 the actual rates are 84 and 44.

The gradual reduction in the number of ectopic points from which extrasystoles arose is also of considerable interest; in Fig. 3 five such points are seen, each represented by different configurations of the electrocardiographic complex. Altogether eight ectopic points were discovered, most of them apparently ventricular in origin; two were seen to arise near the atrioventricular junction but no auricular extrasystoles were noted. In a group of 85 extrasystoles, 56 were apparently coming from the left ventricle. As a result of their heterogeneous origin and their frequency, the hemodynamic factors of the heart were so altered that a continuous irregularity of rhythm occurred. Such an irregularity would come within the definition of auricular fibrillation given originally by Lewis[12] and later by Lamson.[13]

From a clinical point of view, therefore, a diagnosis of auricular fibrillation might be quite justified under such conditions,[14] but at the same time the hazard inherent in digitalis therapy makes this cardiovascular disturbance worthy of some attention. The relative infrequency of strophanthin medication in this country might prevent the clinician from considering this drug as being one of the etiologic factors responsible for the grossly perverted arrhythmia; the attempt at massive digitalis dosage so prevalent at the pres-

ent time among many cardiologists while clinically indicated would unquestionably have caused irreparable myocardial damage and possibly death. Too much emphasis can thus not be laid upon the assistance rendered by graphic methods in the exposition of this otherwise baffling type of cardiac irregularity.

The withdrawal of strophanthin and the supplementary treatment was accompanied by a reduction in the number of hyperirritable foci from which ectopic beats developed. In the time interval between Fig. 3 and Fig. 4 there is a reduction from 8 to 4 such points. In Fig. 5 there are only 2 ectopic foci; their alternation in bigeminal rhythm after the normal cardiac cycle is an interesting example of the fatigue phenomena exhibited by extrasystoles recently described by Roth.[15] Finally, in Fig. 6, the extrasystoles are seen coming from one ectopic focus alone; the disappearance of this last point permitted normal sinus rhythm to be restored.

The rather prompt disappearance of the gross irregularity and the return to normal sinus rhythm through the various stages of triphasic and diphasic rhythms was made possible through the nutritional and therapeutic efficacy of dextrose and metaphylline; we have previously pointed out the rôle of the carbohydrates and especially dextrose in the nutritional disorders of the heart.[16] When such extrasystolic disturbances are due to the ingestion of toxic substances, dextrose when administrated intravenously may be of considerable value without any other additional therapy. This we have had an opportunity of demonstrating in Amanita muscaria poisoning. In an individual suffering from mushroom poisoning with a toxic extrasystolic arrhythmia, intravenous dextrose gave a favorable result.[17]

In the case presented, no especial gastrointestinal symptoms were noted; this stands in contrast to the diarrhea, nausea, and vomiting seen in the first case. Apparently acute strophanthin poisoning is more associated with such gastric phenomena than the chronic types of strophanthin overdosage; this has been the experience of other observers. These two cases are thus interesting in pointing out the difference between the acute and the long maintained reactions to strophanthin overdosage.

SUMMARY

1. Two cases of strophanthin poisoning are recorded; the first is one of acute poisoning while the second is one of chronic self-administered strophanthin overdosage.

2. In both cases, disturbances of cardiac rhythm occurred; these disturbances were extrasystolic in origin and represented a state of hyperirritability engendered in the myocardium as the result of this drug.

3. Withdrawal of the strophanthin preparation resulted in prompt clearing up of the cardiovascular disturbances.

4. The second case is interesting in that it presents an unusually long history of self-administration of strophanthin over a period of four and one-half years during which time about 300 grains of the drug were taken.

5. The withdrawal of the drug was accompanied by a reduction in the number of ectopic points from which the extrasystoles arose; this reduction produced certain unusual disturbances of rhythm.

6. Examples of triphasic and diphasic rhythm are shown and the hemodynamic factors responsible for these phenomena are explained.

7. The intravenous administration of dextrose did much to accelerate the return to normal sinus rhythm.

8. The differential diagnosis of extrasystolic arrhythmia from multiple ectopic foci and auricular fibrillation may be impossible without graphic assistance; the importance of the correct diagnosis lies in the indications for digitalis therapy. Whereas, digitalis is indicated in rapid auricular fibrillation, it is strongly contraindicated in the extrasystolic arrhythmia due to strophanthin overdosage.

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HYDATIDIFORM MOLE AS A CAUSE OF POSITIVE REACTION IN THE ASCHHEIM-ZONDEK PREGNANCY TEST*

By John I. Fanz, M.D., and Edwin S. Gault, M.D., Philadelphia, Pa.

IN OUR work of corroboration of the Aschheim-Zondek Test as a diagnosis of pregnancy, begun February, 1929, we encountered a number of interesting results, which we hope to incorporate in a later paper outlining this work. Outstanding among these, and of sufficient interest to report separately, are our results in a case of hydatidiform mole. Unfortunately we did not learn of the case until after the expulsion of the mole, but recalling from Zondek's work the fact, that the pituitary and ovarian hormones are discharged in detectable amounts in normal cases, until the seventh day after delivery, we determined to ascertain the findings in this instance.

The case itself is briefly outlined as follows: Female, white, age twenty-four, weight 156 pounds, blood pressure 130/74. Married two years, never pregnant previously; last menses occurred August 13, 1928. Early in November, 1928, patient complained of some bleeding from the vagina, with slight pain near Morris' left point. The pain was intermittent and regular. Slight tenderness also present below umbilicus. The physician in charge, suspecting a beginning abortion, placed the patient in bed for a period of three or four days, administering in customary dosage the compound tincture of viburnum. At the end of this period of rest (four days), bleeding ceased entirely, and patient felt well; so well indeed that she resumed her household duties. On April 28, 1929, the physician in charge was again summoned. Abdominal pains were sharp and recurred at fairly regular intervals of thirty minutes. Rhythmic uterine contractions were palpable in a uterus whose fundus did not reach the umbilicus by 2 fingers' breadth. On vaginal examination the mucosae were found congested. Lower uterine segment seemed demarcated; cervix was very soft and boggy, and beginning dilatation was apparent. The breasts showed no signs of pregnancy. After eleven hours of more or less irregular pains (in the eighth month of gestation), the uterine contents were expelled. (See Fig. 1.)

May 5, 1929, bled a little; passed small clot in morning. May 10, 1929, patient developed pain in left inguinal region, exaggerated when she stands up or walks. There is no bleeding. May 14, slight bleeding from vagina increased on May 15. (Menses were due between eleventh and fifteenth.) Pain in left inguinal region disappeared. May 16, bleeding stopped. May 17, again began to bleed for a day and stopped. May 19, bleeding again started. May 21, passed some clots.

June 22, 1929, examination showed retroversion of a soft uterus, enlarged 2X. Cervix negative. Adnexa not palpable. June 28, operation, di-

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Received for publication, April 7, 1930.

latation with graduated bougies and light curettage. Microscopic examination of scrapings revealed hypertrophy of uterine glands of endometrium; stroma is hemorrhagic. No evidence of malignancy. Diagnosis, Hypertrophic endometritis with hemorrhage.

Patient was ultimately discharged July 2, 1929. No bleeding had occurred since curettage (June 28). Cervix was firmly contracted. Uterus is in normal position and mobile. Adnexa negative. General condition good. There has been no recurrence of bleeding. Patient has since become pregnant (last period July 9, 1929) and is probably due April 19, 1930.

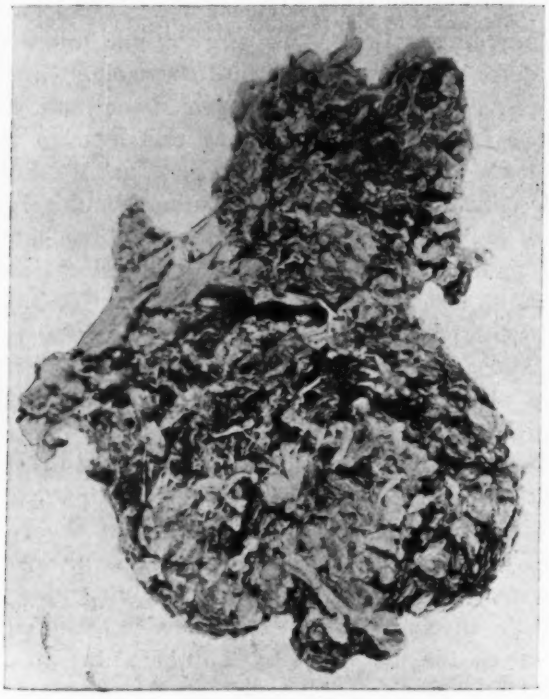

Fig. 1.—Hydatidiform mole. Mass weighed 155 gm, measured 13.5 × 8.5 × 4.5 cm. Note irregular cystic formation, and shaggy fibrinous masses.

The mass (as shown in Fig. 1) was a hydatidiform mole, weighing 155 gm., measuring roughly 13.5 cm. × 8.5 cm. × 4.5 cm. A large amount of blood clot could be removed without breaking the tumor, which was made up of cysts varying in size from 1 mm. to 8 or 10 mm. These cysts are innumerable, walls are thin and translucent, pinkish-white in color, and contained clear fluid. Masses of a fibrinous nature form the groundwork. The suspicion of chorion epithelioma was eliminated, and a diagnosis of hydatidiform mole was established by microscopic study. (See Fig. 2.)

The technic employed in testing was the unmodified method of Aschheim and Zondek. Five immature female mice, each weighing approximately 10 gm., three weeks old, were injected with the urine of the patient, obtained on the third day after expulsion of the hydatidiform mole. On the first day

mouse No. 1 received 0.2 c.c. subcutaneously at 11 A.M. and 5 P.M. Mouse No. 2 received 0.25 c.c. subcutaneously at 11 A.M. and 5 P.M. Mouse No. 3 received 0.3 c.c. at same times. Mouse No. 4 received 0.3 c.c. at same times. Mouse No. 5 received 0.4 c.c. at 11 A.M. and 5 P.M., but died that night. On the following day the 4 remaining mice received by subcutaneous injection doses as shown above, but at 11 A.M., 1 P.M. and 5 P.M. (3 injections being given on second day). On the third day each mouse received respectively subcutaneous doses as indicated, but at 10 A.M. only.

Fig. 2.—Photomicrograph of tumor shown grossly in Fig. 1 (16 mm. objective, 8X ocular). Note groundwork of fibrin and red cells. At center there is an irregular area, bounded by Langhan's cells and syncytium. The center corresponding to villous core is degenerating to cystic formation, although quite cellular. Below and to the left note solid mass of large decidual cells.

Summary of injections and doses is better shown in Table I:

TABLE I

Animal 1 received 6 injections, each 0.2 c.c.
Animal 2 received 6 injections, each 0.25 c.c.
Animal 3 received 6 injections, each 0.3 c.c.
Animal 4 received 6 injections, each 0.3 c.c.
Animal 5 died night of first day.

Animals were killed by illuminating gas and dissected on the fifth day, i.e., two days following last injection.

The following results are summarized and tabulated for brevity.

Fig. 3.—Photograph (Tessar mag. approx. 5X), to show internal genitalia of control mouse, three weeks of age, weight 10 gm. Note slender fallopian tubes. each surmounted by immature ovary, showing no enlargement of follicles nor corpora hemorrhagica (blood spots). See photomicrograph for histology of right ovary, Fig. 4.

Fig. 4.—Photomicrograph (16 mm. objective, 8X ocular) of section of right ovary shown grossly in Fig. 3. Note numerous and immature (small) graafian follicles present. No corpora hemorrhagica (blood spots), nor corpora lutea are present. Entire ovarian section is small.

Fig. 5.—Photograph (Tessar mag. approximately 5X), showing internal genitalia of mouse No. 1 of series, three weeks old, weight 10 gm. Note darker color and greatly thickened tubes and enlarged vagina and ovaries. Note large corpus hemorrhagicum (blod spot) on each ovary. Enlarged follicles are not clearly shown in picture, but were recognizable grossly in fresh specimen. Histologic study confirmed gross findings.

Fig. 6.—Photograph (Tessar mag. approx. 5X), showing internal genitalia of mouse No. 2, three weeks old, weight 9 gm. Note thickening of tubes and enlargement of ovaries. Neither ovary shows corpus hemorrhagicum (blood spot) grossly. See Fig. 7 for histologic picture.

Fig. 7.—Photomicrograph (16 mm. objective, 8X ocular) of section of right ovary seen grossly in Fig. 6. Mouse No. 2 of series. Observe enlarged (matured) character of graafian follicles, some clearly showing ova. Note absence microscopically of corpora hemorrhagica (blood spots), but presence of one large and several small corpora lutea, which have had origin in previous corpora hemorrhagica.

Fig. 8.—Photograph (Tessar mag. approx. 5X), to show internal genitalia of mouse, No. 3 of series, three weeks old, weight 9 gm. Fallopian tubes and ovaries are decidedly enlarged, and are of dark color. Note two large corpora hemorrhagica (blood spots), appearing together on right ovary. Left ovary is negative in this respect. See Fig. 9 for histologic picture.

Fig. 9.—Photomicrograph (16 mm. objective, 8X ocular) of section of right ovary, shown grossly in Fig. 8, mouse No. 3 of series. Note the enlargement (maturation of graafian follicles, some of which show ova). Note large corpus hemorrhagicum (blood spot) at top and to the right. Three large corpora lutea are shown below and to the left. Congestion is marked.

Fig. 10.—Photograph (Tessar mag. approx. 5X), showing internal genitalia of mouse No. 4 of series, three weeks old, weight 10 gm. Fallopian tubes and ovaries are enlarged. No corpora hemorrhagica (blood spots) are present. Histologic study, however, revealed numerous matured graafian follicles, and several corpora lutea in ovaries.

TABLE II

	UNINJECTED CONTROL MOUSE, 3 WEEKS OLD, 10 GM.	MOUSE NO. 1 3 WEEKS OLD, 10 GM.	MOUSE NO. 2 3 WEEKS OLD, 9 GM.	MOUSE NO. 3 3 WEEKS OLD, 9 GM.	MOUSE NO. 4 3 WEEKS OLD, 10 GM.
External Genitalia	Underdeveloped. Vaginal orifice closed. (Typical nymph.)	Fully matured. Vaginal orifice present	Same as No. 1	Same as No. 1	Same as No. 1
Uterus	Not enlarged, pale pink, infantile	Moderately enlarged ++ Pinkish-red	Enlarged + Pinkish	Moderately enlarged ++ Pinkish	Enlarged + Pinkish
Tubes	Not enlarged, thread-like, pale pink	Moderately enlarged ++ Pinkish	Enlarged + Pinkish	Decidedly enlarged +++ Pinkish	Enlarged + Pinkish
Ovaries	Very small, hardly visible, pale pink	Moderately enlarged ++ Pinkish	Enlarged + Pinkish	Enlarged + Pinkish-red	Enlarged + Pinkish
Graafian Follicles (Hist.)	None seen grossly. Histologically very small and numerous	Large, matured, numerous +++	Large, matured, numerous + Some small	Large, matured, Numerous ++ Some small	Very large, matured, numerous + few small
Blood Spots (Grossly)	Rt. ovary 0 Lt. ovary 0 None seen histologically	Rt. ovary 1 large Lt. ovary 1 large	Rt. ovary 0 Lt. ovary 0	Rt. ovary 2 large Lt. ovary 0	Rt. ovary 0 Lt. ovary 0
Corpora Lutea (Hist.)	Rt. ovary 0 Lt. ovary 0	Rt. ovary several Lt. ovary several	Rt ovary several Lt. ovary several	Rt. ovary several Lt. ovary several	Rt. ovary several Lt. ovary several
Interpretation of Results	Reaction—negative grossly and histologically	Reaction—positive grossly and histologically	Reaction—positive histologically	Reaction—positive grossly and histologically	Reaction—positive histologically

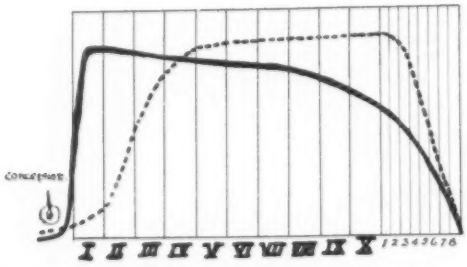

Fig. 11.—(Redrawn from Aschheim and Zondek.) Graphic representation of anterior pituitary and ovarian hormone discharges during pregnancy and puerperium. The ten lunar months are indicated by Roman numerals I to X. The eight days of the puerperium are indicated by Arabic numerals 1 to 8. Heavy solid line (————) is curve of anterior pituitary hormone discharge. Dotted line (- - - - - - - -) represents the variation in ovarian hormone discharge.

Aschheim and Zondek consider a reaction positive with the following findings (due to pituitary hormones): 1. Follicle maturation, blood spots in enlarged and ruptured follicles. 2. Luteinization of follicles even when these occur in but one mouse of the series. We are therefore justified in calling our reaction positive, in that these changes occurred in two mice of the series, as shown in Table II.

The originators of the test attribute the uterine and tubal enlargements, maturation of external genitalia, cornification of vaginal epithelium and clod stage to ovarian hormone. This hormone may be present in the urine of pregnant women in conjunction with the pituitary hormone, or may be produced in and released from the ovaries of the mouse subsequent to the changes produced by pituitary hormones of the patient's urine.

We believe the latter to be the case in our findings.

CONCLUSIONS

A definite positive Aschheim-Zondek test was obtained with the urine of a woman, three days after having expelled an hydatidiform mole. The originators have shown, in several of their earlier works, that the reaction is due to excessive anterior pituitary hormone produced physiologically during pregnancy, and eliminated in the urine in detectable amounts, from the first month until about one week after delivery. As there was in this case no actual fetal formation, with development of chorion and decidua only, we attribute the pituitary hyperfunction to the internal secretion of one or both of these cell proliferates.

On examining the curve of pituitary secretion during amenorrhea of the mother (Aschheim-Zondek, Fig. 11, solid line curve) we find the anterior pituitary hormone rising abruptly in amount during the first month; remaining more or less fixed during the next five lunar months, to fall gradually during the later four months and then more abruptly for a week following delivery. This curve is practically coincident with the development of trophoderm and its derivative, the chorion, and in its later half of declination is coincident with the disappearance of Langhan's cells (from the fifth month on). Can it be that Langhan's cells with their trophodermal precursors through their products stimulate the anterior pituitary body to hypersecretion during pregnancy?

On studying placental development with special reference to syncytium, we find it apparent at the third month, reaching its full development (except as to size) at the fourth month, and continuing its development throughout pregnancy. It seems that syncytial development is practically portrayed by the curve of ovarian secretion (see dotted line curve of chart, Fig. 11) to the extent that it would suggest an effect on the ovarian hormone liberation from the corpus luteum of the ovary.

The decidual proliferate of the uterus can hardly be considered a producer of hormone to the anterior pituitary, due to the fact that it is inherently maternal tissue from the start.

Following this deduction it would seem that the anterior lobe of the hypophysis (pituitary body) acts as a co-secretor organ to the growing placental proliferate (possibly layer of Langhan's) causing anterior pituitary activity rather than the cells of the fetus per se.

Inasmuch as the patient gave no evidence of breast hypertrophy, it would seem that the pituitary hormone has no effect on the breasts of the female. This is in keeping with some unpublished findings of one of the authors, who

found no effect on the lactating and prelactating breasts following administration hypodermatically of pituitrin, during the period of two weeks following labor.

In a further series of experiments we shall endeavor to influence dormant anterior pituitary function by use of placental extract, made from young, four to five months old placentae, rich in Langhan's cells. Work along this line would seem profitable in its application in cases of pituitary hypofunction.

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THE PREPARATION OF SOME BROMINATED OILS AND BROMINATED ESTERS*

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RECENTLY halogenated oils have been used to cast shadows in roentgenology. Work has been done with both iodized and brominized oils but particularly with the iodized compounds. A preliminary report on the use of brominized oils was made in 1926 by Putnam.[1] In the same year Dyroff[2] reported on "Contrastol," a brominized product of German origin. Contrastol is the trade name but no information is given as to its method of preparation. In 1928 Merck & Co. introduced "Bromipin," a 33 per cent brominized sesame oil. The product is described as a bromine addition product of sesame oil containing 31 to 35 per cent of bromine in organic combination.

In the fall of 1926 the following experiment was conducted in order to supply halogenated oil for the roentgenologic examination of gynecologic patients in University Hospital. For this particular work the oil should be opaque to x-rays; nonirritant to mucous membrane; have a low viscosity; be easily eliminated and readily sterilized without the formation of harmful by-products and at the same time reasonable in cost.

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Received for publication, April 2, 1930.

METHOD OF PROCEDURE

The oil to be brominated was placed in a suitable container arranged with a motor-driven stirrer. The temperature was kept below 5° C. and the bromine added directly with constant stirring. The resulting product was washed several times with water and dilute sodium carbonate solution to remove the excess bromine and finally extracted with ether. The following oils were brominated: linseed oil, castor oil, lard oil, Purilla oil, cottonseed oil, rapeseed oil, sesame oil, and peanut oil. Compounds of these oils containing 10, 20, 30 and 40 per cent of bromine were prepared. Preparations containing less than 30 per cent of bromine did not cast well-defined shadows. The viscosity of the oils varied directly with their bromine content. Compounds containing 40 per cent of bromine cast clear-cut shadows. Forty per cent solutions of bromine in linseed, rapeseed, cottonseed, and sesame oils not only cast clear shadows but were of the proper viscosity to pass through a hypodermic needle. Forty per cent bromine solutions in the other oils mentioned were too viscous and could not be used. Forty per cent brominized sesame oil was the best product obtained.

Following the preparation of the brominized oils as described, the fatty acids from rapeseed oil, lard oil and olive oil were prepared and brominated. These brominized fatty acids presented an unsightly appearance and possessed no advantages over the brominized oils from which they were derived.

The methyl and ethyl esters of the fatty acids obtained from rapeseed oil, olive oil, cottonseed oil, linseed oil, and peanut oil were then prepared. The method of preparation of the esters may be described briefly as follows: One part of the oil with two parts of absolute methyl or ethyl alcohol in the presence of 1 per cent anhydrous hydrochloric acid gas was heated on a water-bath under a reflux condensor for twenty-four hours. Barium carbonate was then added to neutralize the free acid and the product washed with water and distilled in vacuo. Upon distillation the esters of rapeseed oil underwent considerable decomposition as evidenced by frothing and bumping. Esters of the other oils were thin, water-white limpid fluids. The esters in all cases proved much more limpid than the oils and the methyl esters were more limpid than the ethyl esters. The methyl esters of linseed oil were the most limpid of all the preparations.

As in the case of the oils the esters were brominated directly rather than in ether solution in order to avoid the formation of undesirable brom ethers. The actual bromination was carried out by keeping the temperature of the reaction mixture below 10° C., the bromine being added slowly and with constant stirring. As soon as the approximate theoretical quantity of bromine had been added there was an appreciable darkening of the solution. Enough ester was then added to the mixture to absorb the free bromine and to dispel the dark color. The bromination was stopped at this point. The brominated products were then dissolved in hot absolute alcohol and the solution allowed to stand until two layers had formed which could be separated. The brominated methyl esters of linseed oil were white crystalline solids, fairly insoluble in ordinary organic solvents. These were discarded as being unsuited for hypodermic administration. The brominated methyl esters from

olive oil, rapeseed oil, and cottonseed oil were more limpid than the ethyl esters from the corresponding oils. For this reason the ethyl esters of the other oils were not brominated.

The brominated methyl esters of cottonseed oil seemed the best suited for the purposes desired. They contained the highest quantity of bromine (42 per cent), were pale amber in color and of low viscosity. When bromine to the amount of 42 per cent was made to react with the methyl esters of the other oils, they became dark in color and were too viscous for hypodermic administration.

Brominated oils decompose very easily when heated. Even at 50° the products darken and thicken. As prepared, the brominated oils and esters are sterile; sterilization being brought about by the chemical processes. If immediately sealed in ampules the products remain sterile. If stored in bottles the products do not remain sterile even in the presence of $\frac{1}{10}$ of 1 per cent of hexyl resorcinol. The pharmacologic properties of these brominated products will be discussed in a subsequent paper.

SUMMARY

1. Methods are described for the preparation of brominized oils and brominized esters of fatty acids occurring in certain oils.

2. The esters were in all cases much more limpid than the corresponding oils.

3. The methyl esters were more limpid than the ethyl esters of the same oil.

4. The color of the product darkened and the viscosity increased as percentage of combined bromine increased.

5. Some brominized esters were solids.

6. The brominized methyl esters of the acids of cottonseed oil contained 42 per cent bromine by weight, were pale amber in color and of low viscosity.

7. Products as prepared were sterile and could be kept so by sealing in ampules.

CONCLUSION

The brominized methyl esters of cottonseed oil are the best suited from a chemical and economical point of view as a halogenated product for roentgenologic work.

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THE EFFECT OF BARIUM SULPHATE UPON THE INCIDENCE OF HUMAN INTESTINAL PROTOZOA*

By Justin Andrews, Sc.D., and Moses Paulson, M.D., Baltimore, Md.

IN 1927, the authors, in a report of the comparative incidences of human intestinal protozoa in defecated and in sigmoidoscopically-obtained specimens of bowel contents, noted (*loc. cit.* p. 1877) that more organisms could be found in specimens from clean bowels than from those which contained barium sulphate, a white powder opaque to x-rays administered to patients preliminary to roentgenologic examination for the purpose of visualizing the gastrointestinal canal. In that series of 40 individuals, the incidence of all protozoan organisms demonstrated in defecated specimens from persons who had not partaken of barium sulphate within six days of the examination was 13.7 per cent whereas the figure for those who had received barium sulphate within that period was 8.7 per cent.

In the last few months, opportunity has been taken to test this observation further using experimental rather than epidemiologic methods. Individuals presenting themselves to the gastrointestinal clinic for the relief of various and sundry complaints referable to the alimentary tract, were given containers with instructions to bring in the following day when they returned for roentgenologic observations, a sample of the next bowel movement, free from urine. Thus a fecal specimen free from barium sulphate was obtained. At their first visit, these same individuals were also instructed, in accordance with the routine of the clinic, to take four ounces of barium sulphate in two glasses of water as preparation for the roentgenologic examination the following day when they received four more ounces at the time of the examination. Due to various reasons, failure to follow instructions exactly, constipation, etc., the first samples were *not* always free from barium.

A microscopic examination, by the simple smear method, of the first sample returned showed the incidence of intestinal protozoa in the group on the basis of a single examination. These findings, with comments upon their significance, are reported in another place (Andrews and Paulson, 1930). The cases positive for protozoa were given further attention. Attempts were made in all instances to get at least one stool a week from these individuals following their barium sulphate ingestion. These specimens were examined microscopically, as in the case of the first stool submitted, to judge whether or not the presence of barium sulphate in the intestine interfered either temporarily or for longer periods of time with the subsequent demonstration of the protozoa which were known to be present when the barium sulphate was taken.

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Received for publication, April 11, 1930.

In all, 312 persons were examined at least once. Of this group 35, or 11.2 per cent were positive for one or more species of intestinal protozoa. Of the 35 positives, it was possible to do follow-up work upon only 25.

Seven of the cases were examined following their routine barium sulphate meal for periods of time varying from three to ninety-eight days. The results of their fecal examinations are expressed in Table I. Briefly summarized, this table shows that following the barium sulphate the number of certain organisms diminished so that they could not be found for periods of time varying from none to twenty-three days subsequent to the barium ingestion. They ultimately reappeared, i.e., in no case did the administration of eight ounces of barium sulphate effect an extermination of the organism.*

TABLE I

EFFECTS OF ROUTINE BARIUM SULPHATE MEAL UPON INTESTINAL PROTOZOA

CASE NUMBER	ORGANISMS FOUND IN STOOL	POSTBARIUM STOOL EXAMINATION		DAYS AFTER BARIUM SULPHATE MEAL	
		PERIOD OF DAYS	NUMBER	NEGATIVE	POSITIVE ON AND AFTER
J 16784	Endameba coli	3	1	3	?
	Trichomonas hominis			3	?
U 21623	Endameba coli	12	3	3	10th day
	Endolimax nana			3	10th day
J 35479	Giardia lamblia	56	7	0	1st day
J 36839	Endolimax nana	31	4	?	5th day
	Iodameba williamsi			?	5th day
	Chilomastix mesnili			?	5th day
J 34917	Endameba coli	75	9	Few cysts seen on 6th day but no more until 27th day	27th day
	Iodameba williamsi			Few cysts seen on 6th and 13th days but no more until 37th day	37th day
J 29905	Endameba coli	41	5	23	30th day
J 34183	Giardia lamblia	10	2	0	1st day

Inasmuch as there was no doubt but that the barium sulphate exercised some reduction upon the numbers of protozoa, it seemed advisable to see if that temporary influence might not be increased and prolonged by extending the period of barium consumption. Accordingly a number of the clinic cases found positive for protozoa were persuaded to take two ounces of barium sulphate in water daily for one week. Their stools were carefully observed microscopically during and after this period in order to estimate the effect of the barium sulphate upon the protozoal infestations. The results are shown in Table II.

It is unfortunate that there was but a single case of Endameba histolytica infection as that particular case seemed to be adversely affected by the barium. Further investigation with this organism is indicated. It is perhaps significant to state that heroic doses of bismuth subnitrate, a similar inert white powder, are sometimes used adjunctive to amebicidal therapy in the treatment of intestinal infections with E. histolytica (James and Deeks, 1924).

*This conclusion assumes that the results in Case J 16784 were not significant inasmuch as it was followed only three days. It seems more than probable that, had the examinations continued, the organisms would have subsequently reappeared.

It was in general difficult to find specimens of Endameba coli, Endolimax nana or Iodameba williamsi during the period of barium consumption, and in a few cases for a short time following. However, in practically all cases the organisms were again detectable some time during the second or third week following the beginning of the treatment.

Trichomonas, in the three instances encountered, apparently disappeared and did not return in the period during which examinations were conducted. This observation should be tested further utilizing cultural methods to check the microscopic findings. It is of especial interest as this organism is the only one of the intestinal flagellates of man which does not produce cysts.

TABLE II

EFFECT OF PROLONGED BARIUM SULPHATE CONSUMPTION UPON HUMAN INTESTINAL PROTOZOA

CASE NUMBER	ORGANISM	POSTBARIUM PERIOD									
		DAYS						WEEKS			
		2	3	4	5	6	7	2	3	4	5
J 31174	Endameba histolytica		−				−	−	−		
J 34054	Endameba coli		−					−	+	+	
J 37819	Endameba coli		−		+			+	+	+	+
J 36939*	Endameba coli	−		−				+	+		
H 21847	Endameba coli		−			−		−	-		
H 11330	Endameba coli		−				+	+			
J 39751	Endameba coli		+				+				
J 29905	Endameba coli		−				+	+	+	+	
U 21623	Endameba coli			−			−	−	+		
H 58975	Endameba coli		+					+			
H 20150	Endolimax nana		−				−	+	+	+	+
J 37945	Endolimax nana			−			+	+			
H 6467	Endolimax nana				+			+			
U 21623	Endolimax nana			−			−	−	+		
J 39182	Endolimax nana			−		+		+	+		
J 31174	Endolimax nana		−			+		−			
J 36839	Endolimax nana		+				+	+	+		
J 39340	Iodameba williamsi		−	−			−	−	−		
J 36839	Iodameba williamsi		−				+	+	+		
J 31174	Iodameba williamsi		−				+	+			
J 39182	Trichomonas hominis			+		−		−	−		
U 21623	Trichomonas hominis				+			−	−	−	−
H 39570	Trichomonas hominis		−				−	−	−		
J 36839	Chilomastix mesnili		+			+		+	+		
H 58975	Chilomastix mesnili		+				−	−			
J 39182	Giardia lamblia			+		+		+	+		
H 6467	Giardia lamblia				−			−	−		
U 11821	Giardia lamblia		+				+	+	+		
J 30049	Giardia lamblia			−				+	+	+	
G. W.	Giardia lamblia	+	+	+	+	+	+	+	+	+	
J. S.	Giardia lamblia	+	+	+	+	+	+	+	+	+	
J 34183*	Giardia lamblia							+	+	+	
J 35479	Giardia lamblia							+	+		
H 39570	Giardia lamblia				−			+	+		

*This individual took two ounces of barium sulphate daily for two weeks instead of for one week.

Chilomastix and Giardia were apparently unaffected by the barium. Giardia in particular showed little tendency to be reduced in numbers even during the first few days after the barium treatment commenced. This may have been due to the fact that Giardia is primarily a parasite of the duodenum, and the vegetative forms, therefore, were not subjected to the condition of

sustained contact with the accumulated barium mass which prevailed in the lower bowel and to which all of the other forms were exposed.

This evidence confirms in general the previous observations of the authors (1927) on the effect of barium sulphate upon the human intestinal protozoa, namely, it causes a reduction in the number of organisms per volume unit of stool as long as it remains within the bowel. It thus reduces the probability of detecting the protozoa by the microscopic examination of a simple smear. It seems improbable that barium sulphate exercises any direct toxic effect upon the organisms, inasmuch as it is chemically inert. The presence of such a finely divided substance accumulated and packed into the colon in a semisolid mass might conceivably interfere with the mechanical activities of the flagellates, and it is obvious that such a substance added to the usual intestinal contents would dilute them. It seems to the authors that this last effect, simple dilution, to a large degree accounts for the characteristic changes noted and for the variability in these changes. If dilution is the effective factor, then it follows that heavy infestations would be least affected, whereas moderate infestations might show a temporary apparent reduction, and light infestations might be rendered indetectable for a longer time. Table II shows instances of prolonged disappearance, transient reduction, and no change at all in the number of organisms found in these cases. Undoubtedly these various results reflect differences in the intensity of the original infestation.

In view of the preceding observations, taken in conjunction with those previously published by the authors (1927), it seems apparent that the presence of barium sulphate in the bowel may at least temporarily interfere with the probabilities of finding intestinal protozoa. Many fecal specimens submitted for microscopic diagnosis from clinic and hospital patients contain large quantities of barium sulphate acquired as the necessary concomitant of roentgenologic examination. Negative reports of protozoologic findings from such specimens should be checked by at least one more subsequent examination made not sooner than six days following barium sulphate ingestion.

CONCLUSION

The ingestion of barium sulphate resulted in such a reduction of the number of intestinal protozoa per volume unit of stool that it was frequently impossible to detect them microscopically. The intestinal amoebae were more conspicuously affected than the intestinal flagellates. The decrease was transient and even extending the period of administration of the barium sulphate did not prolong the effect.

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PHARMACOLOGY OF BROMINATED OILS*

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IN ANOTHER place[1] we have reported on the preparation and properties of brominated oils and brominated esters. Among the properties listed as essential in order that these products might be used for roentgenologic work was freedom from irritating and toxic effects. In 1921 Sicard and Forestier[2] first reported the use of lipiodol as a means of determining the localization of an obstruction in the spinal canal. Lipiodol is a 41 per cent solution of elementary iodine in vegetable oil. In 1923 Sicard, Paraf, and Laplane[3] reported 150 cases in which lipiodol was used with no untoward results. McClaire[4] in 1925 reported one case in which the use of lipiodol was followed by evidence of subarachnoid irritation. Ayer and Mixter[5] in experimenting with iodipin on cats found this substance irritating when placed in the spinal subarachnoid space. Sharpe and Peterson[6] reported three cases in which lipiodol was introduced into the spinal canal. In one case the iodized oil became so irritating that the encysted globules had to be removed by laminectomy five and one-half months afterward. In the other two cases roentgenograms revealed the presence of unabsorbed lipiodol fifteen months after the injection. Sargent[7] in 1923 reported three cases in which lipiodol was successfully used to locate tumors of the spinal cord. In a later paper Sicard and Forestier[8] reviewed their experiences with lipiodol. They report no immediate reactions at the time of injection. Secondary reactions such as headaches, a slight rise in temperature and stiffness in the back occur about six hours after the injection and persist for one or two days. In over 500 patients treated by them there has been no fatal accident. Some of the patients were observed over a period of four years without any symptoms being produced by the retained oil. According to Sicard and Forestier, in the cases reported by McClaire and Sharpe the aggravation of the symptoms occurred after a second operation. Because of this fact they believe the injection of the oil not to be the only causative factor. However, they recommend the removal of the oil at the time of operation.

Not only have iodized oils been used in spinal, cisternal and ventricular injections but their use has been extended as a means of diagnosis in disorders of the chest. The use of lipiodol in relation to chest diagnosis has been quite recently discussed by Nichols.[9] The use of iodized oils is not without contraindications. Nichols particularly mentions hypothyroidism, idiopathic susceptibility to iodine and pulmonary tuberculosis. In addition to these therapeutic drawbacks iodized oils usually possess a higher viscos-

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Received for publication, April 2, 1930.

ity and are less stable to heat than the corresponding bromine compounds. In using brominized oils the possibility of inducing bromism should always be borne in mind.

A preliminary report on the use of brominized oils was made in 1926 by Putnam.[10] In 1929 the Abbott Laboratories introduced brominized esters of the fatty acids obtained from olive oil.

EXPERIMENTAL

Among the first brominized oils studied by us was brominized sesame oil. A series of six rabbits was injected intraperitoneally with 2 c.c. of brominized sesame oil After an observation period of one month in which no symptoms were noted, the dose was repeated. One month after the second dose the animals were killed and a postmortem examination made in each case. In order to test the irritating effects of the oil upon mucous membranes a drop was instilled into the eye of each rabbit twice a week. There was no evidence of conjunctivitis at any time.

The oil was then subjected to clinical tests. Forty-three patients on the service of Dr. P. J. Reel[11] at University Hospital were given intrauterine injections of brominized sesame oil for the purpose of gynecologic diagnosis. In one case there was evidence of irritation discovered later at the time of operation. In some cases the oil was found free in the peritoneal cavity as late as four weeks after the operation. There was no apparent damage to the tissues even in these cases.

Of the brominized esters, the esters of cottonseed oil were found to be best suited for the roentgenologic work, both from an economical and a chemical standpoint. It was found that brominized esters prepared as described in the previous paper[1] were sterile and would remain so if immediately sealed in ampules. However, if allowed to stand unprotected, the preparation soon became contaminated. The addition of hexylresorcinol to the esters in the proportion of one to one thousand did not protect them against contamination. The oil for the following experiment was sealed in ampules immediately upon manufacture. The contents of each ampule were cultured at the time of injection to insure sterility. The pharmacologic effect of the esters was studied when administered by the intratracheal and intraperitoneal routes.

Intratracheal.—Five tenths of a c.c. of sterile oil were introduced into the trachea of a rabbit by means of a hypodermic syringe. The animal's neck was placed in extension and a needle inserted below the isthmus of the thyroid. Another group of five rabbits received 2 c.c. of the oil by tracheal insufflation. A fluoroscopic examination was made to locate the oil in the bronchial tree. Animals which received the oil by the hypodermic syringe showed a slight engorgement of the vessels at the site of injection. There was no evidence in either case of pulmonary irritation, after periods of one, two, and five weeks.

Intraperitoneal.—The effect of intraperitoneal injection was studied from two standpoints, first, the effect of time after a single injection and, second, the effect of repeated injections over a long period of time. Each of a group

of eleven rabbits was injected with 2 c.c. of brominized esters and an hour after the injection the exact location of the oil was determined by fluoroscopic examination. At the end of a week one of the animals was killed and a postmortem examination made. No trace of oil was found, nor was there any evidence of inflammation.

Thereafter at periods of nine, twenty-two, thirty, thirty-six, and sixty-four days one rabbit of this group was killed and a postmortem examination made. On none of these occasions was any trace of the oil found nor any pathologic lesions noted. During the winter months four of the original group receiving a single injection died of pneumonia as verified by postmortem examination. The last member of this group was killed seven months after the beginning of the experiment. Even after so long a period of time there was no evidence of any deleterious effects of the brominized compound.

In a second series of eighteen rabbits the animals were divided into three groups of six each. The members of the first group received an intraperitoneal dose of 2 c.c. For two weeks thereafter daily urinalyses for sugar and albumin were made with no evidence of kidney disturbance. At the end of the two weeks' period the animals were apparently normal in every respect. At the beginning of the third week the animals received 2 c.c. of the brominized esters intraperitoneally per week for four successive doses. Analyses of the urine were made daily to detect any nephritis. In all, these animals received a total dose of 10 c.c. in a period of six weeks. There was no evidence of any harmful effects. Each member of the second group of this series received a single dose of 2 c.c. of the brominized ester. At the end of one week one of the rabbits was killed and a postmortem examination made. At the end of the second week the second rabbit was killed and examined and so on for a period of five weeks. In this manner observations were made as to the effects of a single dose of 2 c.c., after periods of one, two, three, four, and six weeks. One of these animals died from peritonitis forty-eight hours after the first injection but was replaced by a new animal. This case of peritonitis was undoubtedly due to errors in technic. The remainder of the animals showed no ill effects of the oils. On postmortem examination the oils had completely disappeared in every case.

The third group of this series received 2 c.c. of the brominized esters per week for a period of six weeks. At the end of each week one of the rabbits was killed and examined as in group two. In this way the effect of weekly doses of 2 c.c. for one, two, three, four, and five weeks was studied. The last rabbit was killed four weeks after the last injection. This animal had received 10 c.c. and had been under observation ten weeks. The postmortem findings with the exception of the one case of peritonitis reported above showed no member of the entire group injured by the oil.

SUMMARY

Brominized esters of the fatty acids from cottonseed oil and brominized sesame oil were administered to rabbits intratracheally and intraperitoneally. The brominized esters of cottonseed oil were found to be the best for roent-

genologic work both from an economical and chemical standpoint. The oil is eliminated from the site of injection in rabbits within a week.

Repeated doses of the brominized esters produced no deleterious effects.

CONCLUSION

Brominized esters of cottonseed oil may be used in the demonstration of lesions in roentgenologic work.

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DIRECT ISOLATION OF BACTERIUM TULARENSE FROM AXILLARY ABSCESSES*

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IN HIS book *Tularemia*,[1] Simpson states that in only two cases has B. tularense been grown successfully when transferred direct from the patient to culture media, the cultures in those cases being obtained by scraping the walls of the abscesses.

In the two following cases, pus from axillary abscesses, taken soon after fluctuation became evident, produced growths. Smears from the abscesses, stained by Gram's method, showed numerous gram-negative coccoid microorganisms similar morphologically to B. tularense. Cultures taken at a later stage of the disease produced no growths.

The culture medium used for the direct isolation of B. tularense from the patients and for the isolation from the spleens of the guinea pigs used was prepared as follows:

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Received for publication, March 17, 1930.

[1]Simpson, Walter M: Tularemia, New York, 1929, Hoeber, p. 116.

Brain veal infusion agar (P_H 7.6) was heated in the water-bath until the agar was dissolved; 0.05 per cent cystine was added and the heating continued until the cystine was dissolved, then 1 per cent dextrose was added. As soon as the dextrose was dissolved the medium was tubed and sterilized at 15 pounds steam pressure for fifteen minutes. After sterilization the tubes were cooled to 45° to 50° C. and 5 per cent sterile rabbit's serum was added to the tubes. Transfers from the serum medium were made to cystine medium without serum.

The report of the cases follows:

CASE 1.—A. P., colored male, aged sixteen, admitted to hospital, on November 16, 1929, from the City Detention Home, where he had been a prisoner for five months. On admission, the patient complained of headache and pain in left chest. His temperature was 103° F. The physical examination was essentially negative except for a small partially healed laceration (2 cm. long) on flexar surface of third finger of right hand. This lesion had a serous exudate and was not painful or tender. There was little or no reaction in the surrounding tissues. On questioning, the patient said he had cut his finger while washing dishes several days before admission to hospital.

The patient gave the date of the onset of his illness as November 12, 1929, four days before admission, with the following symptoms: headache and general malaise followed by a chill. The symptoms gradually became intensified and were followed by a fever and pain in right chest on the day of admission.

He was put to bed on symptomatic treatment with the impression that the probable diagnosis was influenza. Pneumonia was also considered. The laboratory findings were as follows: normal uranalysis, normal blood picture with no leucocytosis, the nonprotein nitrogen and blood sugar were normal, and the Wassermann reaction negative. Blood cultures taken November 21 were negative. An x-ray film of his chest was reported as negative. The temperature ranged between 101° F., and 102° F., for the first week.

On the nineteenth of November, three days after admission, patient complained of soreness in right axilla. Examination revealed the enlargement of the axillary lymphatics. They were firm in consistency and quite tender. The lesion on his finger had ulcerated and was discharging a semipurulent exudate. Extension of infection was suspected and hot wet dressing put on right hand and fingers. The right axillary gland continued to enlarge and did not seem to be modified by treatment so this was discontinued, dry dressings replacing the wet. The temperature ranged between normal and 100° F. for the second week. The history was carefully checked and the patient, under close questioning, denied any contact with rabbits. A negative Widal was reported on December 1. On the third of December the patient's blood serum was negative for malta fever agglutination but positive for tularemia, the titer being 1:40. The temperature at this time was ranging between 100° F. and 102° F. (third week). A guinea pig was inoculated with material from the lesion on his finger on December 4. The pig died in five days, and on posting showed typical "spotted spleen" of tularemia. B. tularense was recovered from splenic tissues.

The right axillary glands had continued to enlarge and were about the size of an egg with definite fluctuation. An abscess was suspected and this was aspirated with a 17-gauge needle. December 6 six ounces of thick odorless pus was obtained. Two guinea pigs were inoculated with some of this pus, one died on the twelfth day, the other on the thirteenth, and on posting showed typical "spotted spleen." The organism was recovered from the spleens. Also the organism was isolated directly from the patient by inoculating artificial culture media with pus obtained on aspiration of axillary abscess.

The history of the patient was again checked and the jailer of the Detention Home interviewed. He said no rabbits had been used in the institution. On questioning one of the attendants, however, it was found that a rabbit had been caught by dogs on his farm ten miles from the city, and that he had brought it to the Detention Home. The patient had cleaned and dressed the rabbit for him. This informant stated that the patient was taken ill two days later. When questioned again, the patient remembered dressing the rabbit,

though he had denied any contact three or four times previously, but said he was taken ill four days after contact with the rabbit. The incubation period in this case was from two to four days.

The patient became convalescent in the fourth week and was put in a wheel chair. He had no symptoms of the disease at this time. The lesion on his finger had the same appearance as on admission. The right axillary lymphatics were still slightly enlarged and draining through puncture wound, but the tenderness had disappeared. January 7, the patient was entirely symptom-free and had had no temperature for the past four weeks. There was still some drainage from puncture wound in right axilla. Guinea pig inoculations were negative for B. tularense. The patient was discharged on the twenty-first of January in good condition. His last blood serum titer was 1:60.

Case 2.—E. T., white female, aged fifty-two, admitted to hospital on December 26, 1929, complaining of headache, aching pains over body and a generalized weakness. The temperature was 102.2° F., otherwise the examination was essentially negative except for a small ulcerated papule on extensor surface of right index finger and enlarged axillary lymphatics on right. The axillary lymphatics were slightly tender.

The patient gave date of the onset of her illness as December 17, 1929, nine days before admission, with the following symptoms: a chill, followed by fever and vague aching pains all over body. This was followed by a loss of strength with occasional spells of nausea and vomiting. A month before onset, patient injured her right index finger on a board. The finger became infected and had not completely healed when she prepared rabbits. Her son had shot the rabbits while hunting in the vicinity where the rabbit in Case 1 was reported to have come from. Three days following this the patient had a chill.

On admission patient was put to bed on symptomatic treatment with the probable diagnosis of tularemia, based on the history. The routine laboratory findings were completely normal except for a weakly positive Wassermann reaction. The day after admission, December 27, the patient's blood serum agglutinated B. tularense, the titer being 1:800. On the twenty-eighth of December, material from lesion was injected into a guinea pig. The pig died in five days and on posting showed typical "spotted spleen." B. tularense was isolated from the spleen.

During the first week (second week since onset) in the hospital, the patient's temperature ranged from 99° F. to 102° F. Her symptoms were much less distressing. The second week the temperature remained below 100° F.

An enlarged axillary lymphatic gland was removed on the sixteenth of January; this gland was sent to the Bacteriologic Department and B. tularense was isolated from this material by inoculating culture media.

The temperature rose to 100° F. for three days during the third week, but has remained normal now for six days. The patient is symptom-free and the lesion on the right index finger has somewhat of the same appearance as on admission. The axillary lymphatics on right are enlarged and wound where incision for removal of gland was made continues to drain.

On the twenty-fourth of January a large abscess in right pectoral region was aspirated and 120 c.c. of thick yellow odorless pus was obtained. This was injected into a guinea pig and also on culture media. These proved negative for B. Tularense.

Isolation precautions were discontinued on the twenty-sixth of January, and the patient was discharged on the twenty-eighth in good condition. The axillary lymphatics were still slightly swollen, and there was some discharge, but another guinea pig inoculation was negative for B. tularense.

LABORATORY METHODS

DETERMINATION OF COPPER IN BIOLOGIC MATERIAL*

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A NUMBER of published methods for the determination of copper have been tried out in this laboratory. In the colorimetric determination of copper sulphide with buffer solutions,[1] the colors obtained were not satisfactory for comparison and a trace of iron frequently made the results unreliable. The potassium ferrocyanide[2] and potassium ethyl xanthate[3] methods of Scott proved unsatisfactory for our purposes; with the latter, the solutions became turbid and a sediment formed on standing; furthermore, the salt is difficult to obtain pure. Guerithault's method,[4] involving the separation of copper as copper sulphide, was also discarded because of the difficulty of obtaining accurate results with small quantities of copper. Other methods have been proposed: a sulphide thread method,[5] gravimetric method, with KSCN[6]; but none of these has proved practicable in our hands.

We have noted since May, 1926, that it was quite difficult to extract copper from such organs as livers and kidneys, which contain comparatively large amounts of iron, silica, and phosphate, associated with tissues. These required several ignitions at high temperatures after each extraction to make them carbon free. Elvehjem and Lindow[7] faced the same difficulty employing the Pregers method. When carbon free, the copper can easily be plated.

We agree with the suggestion of Redfield, Thomas and Montgomery[8] that the volume should be kept as small as possible (0.2 to 0.3 c.c.) for titrations. When it is diluted to 1 or 2 c.c. the typical color of copper ammonia complex was not produced. The colorimetric methods were discarded because they involved many unknown factors which render the color incomparable with that of the standard. Biazzo[9] proposed determination of copper with pyridine and KCNS in neutral solution with chloroform; here again we noted that presence of ferrous iron produces a yellowish green color and large amount of ferric iron produces a dark brown or dirty brown color. Ferrous salts with pyridine and chloroform produce light green-yellow to light yellow colors.

The electrolytic method seemed most suitable for use with biologic materials on account of the difficulty of removing traces of iron from these substances. When the hydrogen-ion concentration is reduced, other substances such as iron, silica, and calcium, may be deposited with the copper. In such cases a second deposition is necessary: the deposit is dissolved in 10 c.c. of

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Received for publication, March 27, 1930.

concentrated nitric acid and 0.3 to 0.5 c.c. of concentrated sulphuric acid, and the solution is diluted to 200 c.c. in a 250 c.c. beaker. This treatment is required with such substances as oats, lima beans, wheat, carrots, lobsters, bone, and feces.

If the sample is a liquid, such as drinking water, milk, blood, or a vegetable juice, it is measured or weighed and then concentrated to a small volume on a sand-bath or electric hot-plate and taken to dryness in a specially treated porcelain or fused quartz dish or crucible. In the case of a solid, the sample is weighed and dried. The dry substance is carefully ashed in a small quartz dish in an electric oven. All dishes and beakers are previously soaked in a cleaning solution for about twenty-four hours, washed, and then treated with aqua regia to free them from traces of copper.

The ash is ground in a specially treated porcelain or agate mortar, transferred to a 400 c.c. Pyrex beaker, and concentrated nitric acid (sp. gr., 1.42) added, 5 c.c. for a sample of 5 gm. or less, 10 to 50 c.c. for larger samples. The mixture is heated until evolution of chlorine has ceased and then taken to dryness, care being taken to prevent spattering. Fifteen c.c. of concentrated nitric acid is added, a portion being used to rinse out the dish in which the sample was burned, and the mixture diluted from 100 to 150 c.c. It is digested on a sand-bath or electric plate until most of the ash is in solution (forty-five to sixty minutes) and filtered through a dry ashless paper (Whatman No. 42, 9 or 12.5 cm.). The insoluble residue is treated in a fused quartz crucible with 1 c.c. of concentrated nitric acid, ignited, diluted with distilled water, and filtered. It is sometimes necessary to repeat this process several times: to make it entirely free from carbon, the residue, chiefly iron oxide and silica, is finally discarded.

To the combined filtrates are added several drops of concentrated sulphuric acid (sp. gr. 1.84) to keep the iron in solution; any precipitated sulphates are removed by filtration. The solution is evaporated to about 200 c.c. and transferred to a 250 c.c. beaker. The copper is deposited from this solution electrolytically.

For this purpose a platinum gauze (52 mesh, height 2 inches, diameter 1 inch) is used as the cathode and a platinum spiral as anode. These are kept in concentrated nitric acid, washed with distilled water and redistilled alcohol, and ignited to insure a clean surface free from organic matter. They are fastened by means of aluminum screws to a bar of pure aluminum and placed in the copper solution. A current of 3 volts and 1.5 amperes is used for five or six such cells and is allowed to run overnight. In the morning the level of the solution is raised and the electrolysis continued for about twenty minutes to test for completeness of the deposition. The gauze is washed very quickly with distilled water and absolute alcohol and removed before turning off the current. It is dried in an oven for several minutes and weighed. To be certain that all traces of copper have been removed from the solution, a second cone is used and the electrolysis repeated.

The determination may be checked by dissolving the copper from the electrode in nitric acid with the aid of heat and proceeding as follows: if the

copper nitrate solution obtained shows little or no color, it is evaporated to a small volume (sometimes from 0.2 to 0.3 c.c., in order that it may show a blue color with excess ammonia); if the concentration of copper ion is great, the solution is made up to a definite volume and an aliquot portion used. The solution is treated with concentrated ammonium hydroxide until the blue color of the copper-ammonia complex is produced. Glacial acetic acid is then added until the blue color disappears, and then 1 c.c. more. An excess of potassium iodide (1 to 2 c.c. of a 25 per cent solution) is added and the liberated iodine titrated with 0.005 N thiosulphate from a microburette, a drop of freshly prepared starch solution being used as indicator. At the same time a control titration is run, using a solution of copper nitrate obtained by the electrodeposition of the copper from a known quantity of copper sulphate ($CuSO_4.5H_2O$). The accuracy of this method is 0.02 mg.

The determination may also be made by comparing the color of the ammoniacal copper solution[10] obtained above with that of a standard in a microcolorimeter, or with a series of standards made up in sealed tubes of 1 to 2 c.c. capacity as in P_H determinations. Occasionally the tint of color produced cannot be satisfactorily compared in this way.

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TESTING THE EFFICACY OF BACTERICIDAL AND BACTERIOSTATIC REAGENTS FOR USE IN SPECIFIC CHEMOTHERAPEUSIS*

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TESTING the efficacy of chemotherapeutic reagents may be accomplished in several ways. First, on the phenol coefficient basis; that is, by the use of Bacillus typhosus as an indicator. Second, as proposed by Reddish, using Staphylococcus aureus and Streptococcus hemolyticus as representatives of the most frequently occurring infecting microorganisms. Third, by special experimental procedures devised to suit each individual case.

The inadequacy of the first method has been pointed out by many writers. It is well known that while the phenol coefficient obtained by the use of B. typhosus as an indicator may be very high, it may also prove to be of insignificant value when other microorganisms are used.[1] For this reason the phenol coefficient was found to be of little value in practical problems of chemotherapeusis. The second method proved somewhat better. However, in practical work, it too, has been found insufficient The third method remains as the one to which resort may be had for practical chemotherapy. It is frankly admitted that even this procedure possesses disadvantages arising from the lack of a standard for comparison. Yet, it is purposeful and practical insofar as each specific case is concerned.

It was found that S. aureus and S. hemolyticus became acclimatized in varying degree if cultivated on media containing sublethal or subinhibitive percentages of the reagent. The general "cultural history" of the organisms immediately prior to their use as indicators also has an influence upon their resistance to chemical reagents. The age of the culture likewise has a bearing upon the question. It makes a difference whether the culture is newly isolated or one that has been kept as stock for some time.

It was found that even if tested immediately following isolation the resistance of the microorganism to the reagent varied with the source from which it was obtained. This variability is associated with the conditions prevailing at the source. Keilty[2] states that, ". . . antiseptic substances seem to have a selection or specific reaction to the growth of the microorganisms present in a given infection, vice versa, the same organisms present in a series of the same type of infectious conditions will react in their growth differently to the same antiseptic in different cases." The P_H of the fluids, the aerobic or anaerobic conditions, the degree of moisture or dryness of a wound or area of infection, etc., all influence the efficacy of any antiseptic.

*From the Clinical Laboratories and Laboratory Center, Edward Hines, Jr., Center, Hines, Ill. Published with the permission of the Medical Director of the U. S. V. Bureau in accordance with provisions of R & P 6969.

Received for publication, April 15, 1930.

It is evident that the problems of practical and specific chemotherapeusis, where it is dealt with definite clinical cases, are totally different from the problems confronting the regulatory investigator. Unless a given market product makes specific claims on its label it is imperative that the regulatory investigator establish the value of the product as a germicide or antiseptic on a basis comparable with some standard established arbitrarily as a result of practical experience. For instance, three specific "lethal time curves" can be used as the basis for judgment, one each for B. typhosus, S. aureus, and S. hemolyticus. Comparing the "lethal time curves" obtained from the tested material with the "lethal time curves" adopted as standards the regulatory investigator designates the antiseptic superior, inferior, or adequate.

It is impossible in regulatory work to take into consideration specific conditions, since these may be as variable and numerous as the individual cases to which the antiseptic is to be applied. In practical chemotherapeusis, however, the investigator is concerned, not so much with a particular reagent, as with a definite set of conditions under which an infection flourishes. His object, therefore, is to establish analytically the true nature of the conditions and with this knowledge find the reagent required; determine the strength and manner of use; the frequency and length of time it is to be applied in order to effect a cure.

It is evident from the foregoing that the character of the focus of infection must be given first attention. If the infection is of a superficial skin type, stronger reagents and those possessing caustic properties may be included. If the infection is deep or located on a delicate mucosa, the selection will exclude at once those having caustic or irritating properties. In the case of cervical or vaginal infections, cumulative pharmacologic effect must be kept in mind. The possibility of undesirable cosmetic results may have a decided bearing on the selection of a germicide or antiseptic. The experienced chemotherapeutist will have no difficulty, as a rule, in deciding what group of reagents is to be tested for a particular case.

The factor of greatest importance is the etiologic organism. This can be determined by proper bacteriologic methods. All other conditions about the wound must be studied as mentioned above. By subjecting the newly isolated microorganism to the action of varying percentages of the selected antiseptics under conditions simulating those prevailing at the point of infection, choice of a reagent is finally made.

The intravenous method of therapeutic application is much more difficult to approach. In fact, it has been found that no matter what the outcome of in vitro experiments the intravenous method may result in failure. This is the case in bacteremias of the cardiac type. In experiments the etiologic microorganism disappears for a short time to reappear within a few days after the treatment is suspended. Kolmer[3] worked with Pneumococcus and Streptococcus meningitis and states that he has tried intravenous injection of ethylhydrocupreine hydrochloride, mercurochrome, metaphen, gentian violet, the acridine dyes, acriflavine base and rivanol and more recently, the com-

plex Pregl's iodine solution without the slightest effect. He thinks it possible that these substances are helpful in combating toxemias and bacteremias, but are without demonstrable effect on the course and outcome of experimental septic meningitis. He also used the previously mentioned reagents except rivanol in the treatment of human infections. He used ethylhydrocupreine hydrochloride, mercurochrome, metaphen, gentian violet and the acridine dyes, acriflavine and rivanol intraspinally with similar disappointing results in the treatment of experimentally produced infections; also ethylhydrocupreine hydrochloride and gentian violet in human infections without results. So far as the administration of these substances by intravenous or intraspinal injection singly or in combination is concerned Kolmer feels satisfied that nothing is to be hoped for in the treatment of Pneumococcus or Streptococcus meningitis in either man or dog.

In the case of infections of the genitourinary tract, hexylresorcinol was used intravenously. The results lack uniformity not only in the hands of different experimenters but in the hands of one observer dealing with different clinical cases. The difficulty in the intravenous application arises from the fact that no chemotherapeutic reagent has been found that is toxic to microorganisms and inert on the organic constituents of the circulatory fluids.

In simple procedure such as skin application similar lack of consistency is also the rule. Simmons[4] comparing the action of mercurochrome-220 soluble with iodine solution came to the following conclusions: (1) Mercurochrome-220 soluble (2 per cent aqueous) is not effective in disinfection of the unbroken skin. (2) The 2 per cent alcohol-acetone-aqueous solution of mercurochrome and 5 per cent alcohol solution of mercurochrome are slightly better, but these are comparatively feeble bactericidal agents when so applied. (3) The 3.5 per cent alcoholic solution of iodine is more actively bactericidal on unbroken skin than any of the mercurochrome solutions tested. (4) From the standpoint of bactericidal action, tincture of iodine (U.S.P.) was found to be far superior to any of these solutions when used on unbroken skin.

Reddish and Drake,[5] on the other hand, also reported on a comparative study of germicidal efficacy of mercurochrome-220 soluble and U.S.P. tincture of iodine in skin disinfection, concluded that a 2 per cent mercurochrome in aqueous solutions of mercurochrome are efficient clinically in the treatment of wounds and of mucous membranes.

In contradiction to the last conclusion of Reddish and Drake, Rodriguez[6] states that he found 2 per cent mercurochrome-220 soluble to be too feeble an antiseptic to be used safely as a disinfectant of oral mucous membranes. A 5 per cent mercurochrome solution in alcohol and the mercurochrome alcohol-acetone aqueous preparations he found to possess decided advantages over the aqueous solutions. Even these, however, in his experiments failed in too large a proportion of cases to be effective. Iodine of 3.5 per cent and even 1.75 per cent preferably in glycerine, he found effective.

Scott, Hill, and Ellis[7, 8] made a study of the action of mercurochrome and tincture of iodine and concluded that a 2 per cent solution of mercuro-

chrome alcohol-acetone aqueous is as effective in the sterilization of uncleansed human skin as 7 per cent tincture of iodine.

Assuming that in none of the cases cited has there been any definite mental predisposition, the difference in the results obtained can be explained only on the basis of an inherent difficulty in evaluating the bactericidal action of any drug. This difficulty is due primarily to the fact that the experimental conditions under which drugs are tested vary with each investigator.

Two things now become evident to the practical chemotherapeutist: (1) For the present all application of an intravenous character, except, of course, in syphilis and the like, must be suspended until experimentation develops more hopeful prospects. (2) In the field of infections of the mucosa the problems must be solved as befits each individual case.

A generalized plan, however, may be outlined which is hoped will prove of service. The conditions at the focus of infection determine at once the group of reagents to be given tentative consideration. For example, sensitive open wounds and infected mucosae exclude the use of any reagents possessing caustic or generally irritating properties, such as bichloride of mercury.

The isolation of the etiologic organism then follows. The test organism is obtained directly from the focus of infection. This is essential since, as stated previously, it has been found that the age of the culture, its "culture history," and the source of isolation are factors influencing the resistance of the microorganisms to the reagents.

The next step is to determine the conditions under which the microorganism is to be tested. These must simulate the conditions existing at the focus of infection. In the case of an open and bleeding wound, the procedure will be as follows: Blood agar slants of a P_H reaction the same as the fluids of the wound containing varying percentages of the chemotherapeutic reagents under consideration are made. Streaks of the culture of the newly isolated microorganisms are then made in duplicate upon agar slants and incubated at 37° C. for twenty-four hours. As control a streak upon blood agar free from any of the reagents is also made. If possible, it is preferred that the blood used in the culture of the microorganisms be obtained directly from the case under treatment. The reagents completely inhibiting growth in the highest dilution are selected for final testing, while the less effective may be retained for further observation.

Final testing is carried out as follows: A twenty-four hour blood broth culture of the organism is prepared. Varying percentage dilutions of the reagents in saline, having the same P_H reaction as the infected focus and containing the same concentration of blood cells as the blood agar slants, are prepared. To 10 c.c. of the solutions thus made, 1 c.c. of the twenty-four hour suspension is added, thoroughly mixed, and a 1 c.c. transfer made into another tube of similar solution. The second tube is again mixed and a third transfer made. In the first tube there will be 10 per cent, in the second 1 per cent, and in the third 0.1 per cent of the broth culture suspension. The tubes are now incubated at 37° C. and 0.5 c.c. of the solution removed at

intervals of five, fifteen, thirty, and sixty minutes and placed into tubes of clear sterile broth and incubated at 37° C. for twenty-four hours. The drug that shows complete inhibition of growth in the broth tube at the end of forty-eight hours in the greatest dilution is considered appropriate for use.

Cognizance is taken of the possible bacteriostatic effect due to traces of the reagents. However, in practical chemotherapeutic work such as is dealt with here, bacteriostatic results are as effective and desirable as bactericidal, since if kept in check only by bacteriostatic influence, the infecting organism has no chance to multiply and is finally eliminated through tissue reaction. Another factor influencing the chemotherapeutic progress of accessible foci of infection, and which is omitted in regulatory testing and in most of the experimental work on disinfectants, is the influence upon the reduction of the number of infecting organisms accomplished by mechanical means incident to frequent swabbing, douching, washing, and brushing.

If, however, it is desired to know whether the inhibition of the growth is due to complete killing of the microorganism or merely the bacteriostatic effect exerted upon it by the traces of the reagent, blood agar plate transfers are made from the broth tubes in 1.0 c.c., 0.5 c.c., 0.1 c.c. amounts. If the plates remain sterile after forty-eight hours' incubation, it is considered that the effect of the drug was bactericidal. If the blood agar plates develop a bacterial growth at the end of forty-eight hours, the effect of the drug upon the particular microorganism is merely bacteriostatic.

In cases where the material covering the focus of infection is not bloody, but is heavy and mucopurulent, a slightly different procedure is adopted. Agar plates containing from 0.1 per cent to 0.5 per cent of noncoagulable exudative material in the same strength as used on blood agar plates are added to the saline-reagent dilutions. The remainder of the procedure is the same as above. The mucopurulent material developing in and around the focus of infection has a decided effect upon the efficiency of the germicidal reagent. In practical chemotherapeusis this effect is considerably minimized and oftentimes entirely eliminated as noted above by the frequency of the mere mechanical application.

Where the mucoid material is plentiful, persistent, and of semicoagulated character, and it becomes desirable to determine the penetrability of the germicidal reagents, the following procedure is resorted to: Pieces of the mucopurulent material double the size of an ordinary bacteriologic needle-loop are placed carefully into varying percentages of saline-reagent solutions free from blood or exudative material, but having the same P_H as the material itself. They remain in the solutions at 37° C. for periods ranging from five to sixty minutes. At definite intervals the pieces are carefully washed in sterile pure saline to remove traces of the drug. They are then placed into sterile and clear broth tubes, thoroughly broken up and emulsified in the broth and incubated for twenty-four and forty-eight hours. The strength of the disinfecting drug or reagent which sterilizes the mucopurulent material, if not too strong for the tissues affected, is suitable for occasional application. This does not, however, exclude frequent applications of the weaker solutions.

It is well known that certain dyes are highly poisonous even in the minutest quantities.[9] Chemicals generally recommended for internal administration may prove poisonous to some individuals. This is particularly true of the drugs and chemicals which have in their structure an amino group. The most outstanding example of this kind is furnished by hexamethylenetetramine. This drug is recommended by the U.S.P. for internal administration in cases of urinary infection. Yet in some cases it causes hematuria and dermatitis. Reports of such cases are numerous in the medical literature.[10, 11, 12] The question of individual idiosyncrasy to drugs has now become a prominent one, and it is difficult, indeed, to foretell the secondary effect any drug may have upon a patient. It is, therefore, suggested that a proper skin sensitization test be performed upon the patient before final recommendation for specific application is made, and that the laboratorian who studies chemotherapeusis have free access to the cases under consideration as a matter of scientific accuracy and safety.

Four case reports are cited:

CASE 1.—Vaginal chemotherapeusis.

Material from cervical canal was received on swab and studied by the procedure outlined. Etiologic organism, streptococcus possessing weak hemolytic properties. Acriviolet, gentian violet, mercurochrome, and methylene blue were tested, with the results as follows:

Acriviolet 1-10 000 for sixty minutes. 0.5 c.c. transferred to broth and incubated for forty-eight hours. Inhibition of growth complete.

Gentian violet	ditto
Methylene blue	ditto
Mercurochrome	growth
Control	growth

Daily application of gentian violet in 1-100 dilution in saline was recommended in addition to usual douches. Condition cleared in two weeks.

CASE 2.—Culture from skin condition (cellulitis).

Etiologic organism: hemolytic streptococcus.

The following reagents were tested:

Acriviolet 1-10 _ _ for sixty minutes. 0.5 c.c. transplanted to broth and incubated for forty-eight hours. Inhibition of growth complete.

Acriflavine	ditto
Gentian violet	ditto
Mercurochrome	ditto
Methylene blue	ditto
Control	growth

Frequent and prolonged bathing of affected area, intermittently in 1-1000 dilution of acriviolet and gentian violet was recommended. Condition cleared within five days.

CASE 3.—Slow healing wound on left forearm.

Dark-field examination: negative.

Complement fixation and precipitation tests of blood serum, negative.

Etiologic organism, Streptococcus pyogenes, nonhemolytic.

Gentian violet 1-25 000 completely inhibited the infecting organism.

Frequent local applications of gentian violet 1-250 dilution in saline was recommended. Condition cleared within two weeks.

CASE 4.—Slow healing wound on left leg.

Examination of fresh material shows the presence of acute purulent exudate, heavy pus, and necrotic cells.

Etiologic organism: Streptococcus pyogenes, nonhemolyticus.

Gentian violet and acriviolet 1-5 000 inhibited the growth of this organism.

Daily application of 1-200 gentian violet (AM) and 1-200 acriviolet (PM) was recommended.

Wound nearly closed in three weeks.

Grateful acknowledgment is made to Dr. L. H. Prince, Pathologist at this Station, for his kind assistance in the preparation of this manuscript, and to Dr. Sinclair S. Levine of the Prospect Hospital, Hartford, Conn., for applying the above outlined chemotherapeutic procedure and for permission to publish briefs of some of the cases.

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THE ACID-FAST QUALITY OF BLASTOCYSTIS HOMINIS*

By Robert A. Keilty, M.D., Washington, D. C.

THE purpose of this presentation is to note the acid fast quality of Blastocystis hominis in the feces. This protozoa is very commonly met with in the routine examinations of feces. According to Wenyon there is some question as to whether this organism belongs to the bacterial or the protozoal group.

I am in the habit of staining a slide for the tubercle bacillus in the routine examination of most all material including feces. I use a method which I reported several years ago in which 30 per cent hydrochloric acid followed by alcohol is used as the decolorizing agents. Ever since the criticism of Rosenberger's work in the blood, a study which I have always felt fortunate and honored in having taken part in, I have been on the track of all possible acid staining bodies or artefacts other than the tubercle bacillus.

In feces there are often encountered acid fast globular masses in stained preparations. Recently I have recognized some of these as Blastocystis hominis. In the acid fast stained slide the central hyaline-like body of the organism retains the red stain while the granular surrounding bodies in the clear ectosac are not acid fast but appear as blue dots. These bodies vary in size as they do in the fresh slide, are somewhat shrunken by the drying and fixation. The larger they are the more intensely they take the red stain and presumably the more acid fast material they contain.

I have not made an exhaustive search of the literature but I am not familiar with any previous report of the acid fast quality of this particular organism.

CONCLUSION

Blastocystis hominis, a fairly constant protozoon found in the feces, not always but frequently when stained as for the tubercle bacillus shows the clear hyaline central body definitely acid fast in character. This could never be mistaken for the tubercle bacillus but its recognition as such rules out an annoying so-called artefact in the routine examination of the feces for the tubercle bacillus.

*Presented from the Mary A. Keilty Research Memorial.
Received for publication, February 26, 1930.

SIMULTANEOUS DETERMINATION OF INORGANIC PHOSPHATE, SUGAR AND LACTIC ACID IN BLOOD*

By Sergius Morgulis, and Sherman Pinto, Omaha, Neb.

IN CONNECTION with our work on blood glycolysis it was necessary to develop an analytical procedure which would permit the simultaneous determination of inorganic phosphate, glucose and lactic acid on the same blood sample. A very important consideration was the economic use of blood for this purpose. A unified system of analysis which allows the utilization of a limited amount of material for a variety of determinations is, of course, always very desirable, but the adaptation of analytical methods for this purpose often requires much preliminary experimentation to secure the proper conditions for satisfactory results. The procedure which we have developed with the aid of standard methods permits one to carry out all three determinations in duplicate with a single sample of 3 c.c. of blood. The selection of a proper protein precipitating reagent was essential since the blood filtrate had to be suitable for all three analyses. We found that trichloracetic acid answered this purpose fully. One volume of whole blood or of defibrinated blood and nine volumes of 7 per cent trichloracetic acid are mixed thoroughly and allowed to stand a few minutes. We generally make the precipitation in a large centrifuge tube, removing the clear supernatant liquid after the contents have been centrifuged.

For the inorganic phosphate determination the method of Kuttner and Cohen[8] was chosen because of the small quantities required for the analysis. The results obtained by this method are very reliable provided the tubes in which the reaction is carried out are carefully calibrated.

It is convenient to employ two standards, so that if the phosphate content of the unknown solution is very high or very low, it can be quickly determined. Standard A is made to contain 0.0025 mg. and Standard B, 0.005 mg. of phosphorus. These two standards are made by suitable dilution of a stock standard containing 0.1 mg. of phosphorus per c.c. We found the Bausch and Lomb microcolorimeter, with one c.c. cups, very suitable for this determination, instead of using the special colorimeter described by Kuttner. Several test tubes 7.5 × 1 cm. should be accurately calibrated to contain one and two cubic centimeters. The tubes calibrated at 2 c.c. are to contain the standard, and those calibrated at 1 c.c. the unknown solution. Transfer to the tube graduated at 1 c.c., 0.5 c.c. of the trichloracetic acid filtrate. This must be accurately measured, and is equivalent to 0.05 c.c. of blood. Add 0.4 c.c. of the molybdic-sulphuric acid solution, and mix by sharply tapping the lower end of the tube. The contents of the tube are diluted with the

*From the Department of Biochemistry, University of Nebraska College of Medicine. Received for publication, April 5, 1930.

diluted stannous chloride reagent *exactly* to the 1 c.c. mark, and mixed as before. The optimum color appears within fifteen seconds. The two standards, to be prepared at the same time as the unknown, are made as follows: to one tube graduated at 2 c.c. is added 1 c.c. of Standard A, and to a similar tube 1 c.c. of Standard B. Then 0.8 c.c. of the molybdic-sulphuric acid solution are added to each tube, the contents being well mixed, and finally made up to the mark with the diluted stannous chloride reagent. The unknown is compared in the microcolorimeter with the standard which more nearly approximates it in color. Satisfactory readings are obtained if the standard is set at 20 mm. The phosphorus content of the unknown solution is then calculated from the formula:

$$\frac{50 \text{ (or } 100)}{\text{Reading of unknown}} = \text{Mg. P per 100 c.c. blood.}$$

The selection of a suitable procedure for the sugar determination was associated with much greater difficulty. The Schaffer-Hartmann method was not applicable owing apparently to the formation of some compound between the trichloracetic acid and the copper reagent. The results by the Folin-Malmros procedure were too erratic. We finally succeeded with a modification of Hagedorn-Jensen's well-known sugar method.[5, 6]

It is convenient to carry out the entire determination in a single test tube 3 × 11 cm. To 1 c.c. of the trichloracetic acid blood filtrate are added 5 c.c. of 0.067N NaOH to neutralize the excess acid. An indicator must not be added as this affects the results materially, giving too high sugar values. Likewise, Na_2CO_3 cannot be used for the neutralization as this gives invariably low results. Upon adding 2 c.c. of the alkaline potassium ferricyanide solution the tube is placed immediately in a boiling water-bath for fifteen minutes because, if much time is allowed to elapse between the addition of the alkaline ferricyanide and the heating, erratic results will be obtained. Cool, add 3 c.c. of the iodide-sulphate solution, then 2 c.c. of 3 per cent acetic acid solution and titrate from a microburette with 0.005 N sodium thiosulphate, using as an indicator 2 drops of 1 per cent soluble starch in saturated NaCl solution. The end-point should be taken as the point of disappearance of the last trace of color due to starch-iodine. The sugar concentration may be calculated from the tables prepared by Hagedorn-Jensen,[5] or it may be calculated as follows:[1]

(B − E) × 0.177 × 100 = mg. reducing substances per 100 c.c. blood, where B = c.c. of 0.005 N $Na_2S_2O_3$ required for the blank, corrected by the normality factor, and E = c.c. of 0.005 N $Na_2S_2O_3$ required for blood, also corrected by the normality factor.

We were able to get results by this method within about one per cent of the theoretical amounts, provided the determination was made on solutions of no less than 40 mg. per cent glucose concentration. As the sugar concentration fell below this limit, the results became progressively less satisfactory. With 38 mg. per 100 c.c. we already failed to recover 10.5 per cent: and this error increased to 37 per cent as the glucose concentration was reduced to about 29 mg. per 100 c.c. Likewise, the method was not applicable to glucose concentrations above 200 mg. per 100 c.c. When in the

course of glycolysis the sugar concentration drops very low, a larger amount of the blood filtrate must be employed to analyze a quantity corresponding to not less than a 40 mg. per cent concentration. A blank with the greater concentration of trichloracetic acid must, of course, also be run.

The low results obtained with the smaller sugar concentrations are not, however, due to an intrinsic defect of the method. By substituting a 0.002 N thiosulphate for the regularly employed 0.005 N solution for the titration small quantities can be determined with the same degree of accuracy as the larger amounts of sugar. It is really a matter of choice of either keeping two thiosulphate solutions on hand or doubling the quantity of blood filtrate for the analysis.

The lactic acid was determined on the trichloracetic blood filtrate by Friedemann's procedure,[2, 3] by which 98 per cent of the acid present is recovered over a range of concentrations to be expected in normal or glycolyzed blood. Twenty c.c. of the filtrate are transferred to a 100 c.c. volumetric flask and to this are added 10 c.c. of a 20 per cent calcium hydroxide suspension. This is followed by 10 c.c. of a 20 per cent copper sulphate solution. The mixture is shaken frequently for thirty minutes and is finally diluted to the mark with water.[2, 9] After thorough mixing the contents are centrifuged, usually one minute at 2000 r. p. m. being sufficient, and the supernatant liquid is poured through a hardened filter paper into a flask. Approximately 85 c.c. of filtrate can be recovered in this manner. It is essential to use a hardened filter paper since a scum forms on the centrifugate which causes erratic results in the subsequent analysis unless it is removed. Forty c.c. of the filtrate equivalent to 0.8 c.c. of the original blood are used for an analysis, so that the determination can be made in duplicate. The apparatus used is that described by Friedemann, Cotonio, and Shaffer.[3] The 40 c.c. of filtrate are measured into the distilling flask, and followed by 5 c.c. of 2M H_3PO_4. The calcium salt, which precipitates, dissolves on shaking. Ten c.c. of 10 per cent $MnSO_4$, a pinch of talcum and enough water to make 100 c.c. are then added. We have found 0.005 N $KMnO_4$ a satisfactory oxidizing agent if it is added slowly, so that approximately ten minutes is used in oxidizing the lactic acid present.[4] When the oxidation is completed, the contents of the tower are washed into the receiving flask with five to seven 5 c.c. portions of water, and on adding 0.5 c.c. of 1 per cent starch solution in saturated NaCl, the excess bisulphite is titrated with 0.1 N iodine solution. The excess iodine is neutralized with one drop of 0.1 N sodium thiosulphate, and the color of the solution is adjusted to a delicate blue with the dilute iodine used in titrating the "bound" bisulphite. This is decomposed by adding 10 c.c. of a saturated sodium bicarbonate solution, and the liberated bisulphite is titrated with 0.002 N iodine.

For the calculation of the analytical results the c.c. of 0.002 N iodine, corrected by the normality factor, are multiplied by 1.25, since 0.8 c.c. blood were used for the determination. From the product the titration value of the blank is to be subtracted. While a blank on the trichloracetic acid in an amount equal to that in a blood filtrate analysis has usually a small value, upon treating the trichloracetic acid with $CuSO_4$ and $Ca(OH)_2$, as is done in

the removal of sugar from the blood filtrate, the blank titration value is increased. In many determinations we found that the average value of a blank so treated is 1.07 c.c. of 0.002 N iodine. Since the extra bisulphite binding substance is formed in the treatment with $CuSO_4$–$Ca(OH)_2$, it seems that either the trichloracetic acid or possibly some impurity associated with it (dichloracetic acid?) gives rise to substances responsible for the larger blank. This, of course, is very essential to bear in mind, as otherwise very erroneous results might be obtained. The product, c.c. 0.002 N $I_2 \times 1.25$, minus the blank, multiplied by 9 gives the mg. lactic acid per 100 c.c. blood, since each c.c. of the iodine solution corresponds to 0.09 mg. lactic acid.

In our experience the use of mercuric nitrate as the protein precipitant in the lactic acid determination is not as desirable as the trichloracetic acid. Apart from the fact that the procedure involves many more operations and actually takes up much more time, the results so obtained are uniformly lower than by the trichloracetic acid precipitation. Whereas with the trichloracetic acid we recovered on the average 97.5 per cent of the theoretical amount of lactic acid, the results with the mercuric nitrate were very much less satisfactory. In our hands at any rate, the use of the mercuric nitrate as precipitant gave a yield of only about 85 per cent of the theoretical lactic acid present.

We give in Table I some glycolysis experiments in which all determinations were made in duplicate on 3 c.c. samples of blood.

TABLE I

GLYCOLYSIS OF DOG BLOOD

TIME, HOURS	MG. P.	MG. SUGAR	MG. LACTIC ACID
0	4.75	103	21
2	4.78	69	60
4	4.50	47	85
7	7.15	21	120
0	5.95	111.5	30.5
2	5.40	69.5	67.0
4¼	4.65	38.0	100.0
6	5.20	23.5	105.0
8¼		21.5	107.5

SUMMARY

A procedure is described for determining simultaneously inorganic phosphate, glucose and lactic acid on a trichloracetic acid filtrate of blood. The analyses can all be made in duplicate, only 3 c.c. blood being required for all the determinations.

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AN ELECTRICAL DEVICE FOR GRINDING TISSUE UNDER ASEPTIC CONDITIONS*

By Harry O. Maryan, B.S., M.D., Chicago, Ill.

THERE are various methods for grinding tissue under sterile conditions. The most common of these is the mortar and pestle method and its various modifications. Latipie[1] in the early part of the nineteenth century devised a hand tissue grinding apparatus which ground the tissue so fine that it could be injected into animals. This method was adopted by Charcot[2] of the Pasteur Institute for the study of cancer and also by Carrel[3] for the study of filterable viruses. Hirschfelder[4] devised a small hand tissue grinder, which was modified by Rosenow, and this was also improved by Nickel[5] in 1929. The objection to all of these methods is that they are tedious and offer unavoidable sources of contamination.

Rosenow[6] in 1914 described his sterile air chamber containing a mortar pestle and sand, thereby reducing some of the sources of contamination. During the later part of the same year he further developed the sterile air chamber containing the mortar, pestle, and small meat grinder.

The thorough grinding of tissues helps one to isolate organisms if present in their depths. This was definitely shown by Rosenow's[7, 8] work on crushed lymph nodes of arthritis deformans. In one series of 38 cases of arthritis deformans, he isolated organisms in 35 cases; in another series of 54 cases he isolated organisms in 47 cases. In a third series of 12 cases of acute articular rheumatism, he isolated organisms in all. Paston,[9] fifteen years later using the same type of crusher and a similar technic, isolated organisms from the lymph glands of patients with infectious arthritis in 72 of 120 glands.

After using Rosenow's hand crusher and sterile air chamber combined with the meat grinder, I found them unsatisfactory in the crushing and grinding of fibrous tissue. The main difficulties were the unavoidable sources of contamination, through handling, and the great difficulty of crushing fibrous tissue into a fine enough state. Therefore, after much experimentation I developed a motor driven tissue grinder which was especially effective in grinding fibrous and elastic tissue, under aseptic conditions. This new device has several advantages over the older forms of grinders.

*From the Department of Obstetrics and Gynecology, College of Medicine, University of Illinois.

Received for publication, April 25, 1930.

1. It allows the operator the use of both hands for the manipulation of the material to be ground.

2. It expedites the grinding operation of fibrous and elastic tissue, thus improving the technic, the entire operation takes about three or four minutes.

3. It reduces the sources of accidental contamination to a minimum.

DESCRIPTION OF APPARATUS

The apparatus* consists of a one-tenth horse power universal motor, and a sterile air chamber with a small detachable meat grinder. The air chamber containing the meat grinder is mounted on a metal base by means of a grooved sliding track, the size of which is nine inches long and six inches wide. In

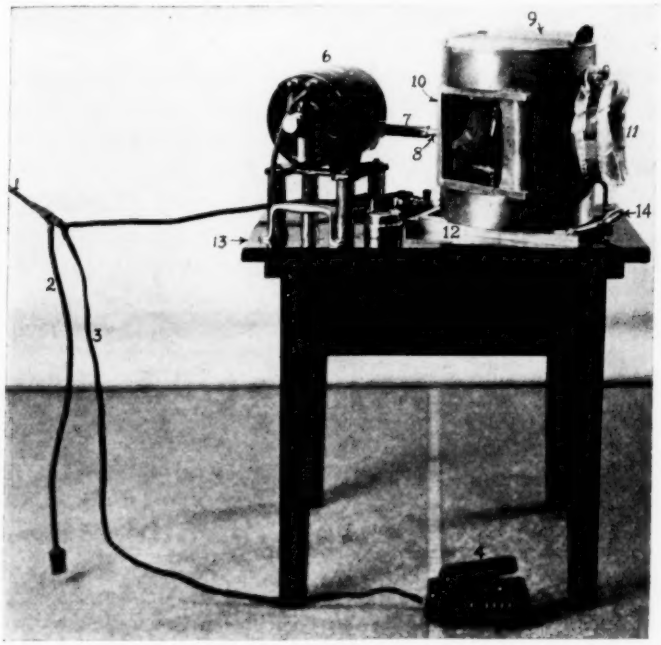

Fig. 1.—(*1*) Main Cord. (*2*) Cord for direct connection. (*3*) Cord connecting foot rheostat. (*4*) Foot rheostat. (*5*) Switch. (*6*) 1/10 universal motor with gear reducing box of 10:1 ratio. (*7*) Sleeve coupling. (*8*) Shaft of meat grinder. (*9*) Sliding framed window of sterile air chamber. (*10*) Side sliding door. (*11*) Circular front opening with attached moleskin glove by band clamp. (*12*) Sliding grooved track of base and can. (*13*) Metal base. (*14*) Handles attached to air chamber.

this way the air chamber is connected to the motor and can be dismantled for sterilization. The motor has a gear reduction of ten to one in order to reduce the speed from 3000 r.p.m. to 485 r.p.m. This increases the pull at the shaft to about ⅘ of a h.p. By means of a foot rheostat the speed can be controlled to as low as 100 r.p.m. The upper limit of speed was found necessary for grinding fibrous tissue, and the lower speeds were adequate for grinding soft tissue.

The air chamber is a vertical tubular air-tight can nine inches long and eight inches in diameter, constructed from ⅛ inch sheet brass, the seams of

*Manufactured by V. Mueller & Co., Chicago, Ill.

which are brazed. There is a 4½ inch circular opening in the front, surrounded by a one inch rim which is brazed to the chamber and to which is attached a long fleece-lined canvas glove, by means of a ½ inch band of brass with clamp and screw. This makes the connection between the glove and collar air tight. On the top of the chamber is a rectangular opening 6½ inches long by 4¾ inches wide surrounded by a three sided frame brazed to the top, into which slides a framed heavy glass. In the left wall of the chamber is a similar opening three inches long by five inches wide into which a grooved three-sided frame is brazed, and into this slides a heavy brass door. On the inside of this opening there are two vertical grooved slides brazed to the wall, in which a brass door two inches high by three inches wide slides. These sliding doors control the size of the opening both horizontally as well as vertically, thereby making the opening as small as two inches high to three inches wide for the introduction of the tissue. The larger opening allows the test tube or beaker to be inserted without undue angulation for the transferring of the tissue. On the inside of the bottom of the chamber, and one inch from the back wall is a circular one inch rim 3¼ inches in diameter which holds the mortar.

The height of the meat grinder is 6⅛ inches with a support of 3½ inches long. The barrel is 3 inches long and has a diameter of ⅞ of an inch, with multiple 1/16 inch openings in the nozzle. The hopper is 2 inches high with diameters of 1⅝ inches by 2⅞ inches. The worm with the shaft is 4⅛ inches in length. The shaft passes through a ⅝ inch circular opening in the middle of the posterior wall which is surrounded by a ⅛ inch rim, and fits into a ½ inch sleeved coupling that comes off the shaft of the motor. The meat grinder is fastened to the posterior wall by three thumb screws which pass through three 1/16 inch holes in the shaft.

STERILIZATION

The air chamber with its attached glove, mortar, pestle instruments, and small bottle of sand are sterilized in a dry heat oven at a temperature of 150 to 210° C., for one or one and one-half hours. The brazed brass construction was chosen so that it would withstand very high temperatures without warping or rusting. This was one of the objections to the use of other air chambers. The sleeve of the glove should be thoroughly moistened before sterilization so as to prevent charring.

TECHNIC

1. Desired tissue is excised under aseptic conditions and is seared through top of a bunsen burner flame.

2. The door is flamed before opening and closing and the tissue is inserted through small opening into the mouth of the meat grinder by means of large tissue forceps and the door is then closed.

3. With the gloved right hand the mouth of the grinder is filled with sand and the tissue is held in place by means of a probe or tissue forceps.

4. The current is then turned on by switch or foot rheostat, and the tissue is ground and forced through small opening in the nozzle of grinder; it

then falls into the mortar below. If entire ground tissue is not expelled, add more sand and grind again.

5. The fluid media is poured through the large side door with the left hand into the mortar containing the ground tissue and sand, so that a thorough mixture can be made.

6. The ground tissue by means of a small spatula, which is held in the gloved right hand, is transferred into the desired media through large side door and the door immediately closed with the left hand and flamed. The gloved hand always remains in place.

a. Right hand is withdrawn partially into the sleeve of glove, which holds the base of the beaker.

b. Cotton plug is flamed and removed with left hand, and beaker is transferred to the right hand.

c. The side door is opened with left hand, and the bottom of beaker is placed just below the base of the opening, so that the neck is properly angulated within the air chamber. The gloved right hand with a small spatula transfers the ground tissue into the beaker.

d. Beaker is transferred to sleeved right hand, door is closed with left hand, neck flamed, and cotton plug is inserted with left hand.

Investigators have shown that the crushing and grinding of tissue does not kill the organisms; it yields just as good a culture as that obtained with the mortar and pestle but with less danger of contamination.

SUMMARY

1. The electric tissue grinder with especially designed air-tight chamber grinds soft tissue, as well as fibrous tissue. It reduces the sources of unavoidable contamination to a minimum, and is very expedient.

2. The technic employed is not cumbersome or tedious.

3. The tissue is ground under the most ideal aseptic and atmospheric conditions.

4. Tissue culture enables one to isolate the organisms in its depths.

5. The most common methods employed for tissue culture are:

a. The mortar pestle and sand.

b. Latipie's hand tissue grinder.

c. Various types of hand tissue crushers.

d. Rosenow's air chamber with meat grinder.

6. The organisms are not killed by the searing, crushing, or grinding of the tissues involved in this technic.

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A NEW HEMOGLOBINOMETER*

By Russell L. Haden, M.D., Cleveland, Ohio

AN ESTIMATION of the hemoglobin content of the blood is one of the most valuable of laboratory examinations and should be one of the simplest. Very accurate determinations can be made by the oxygen capacity method, with the spectrophotometer, or from the iron content. None of these procedures, however, is simple enough for routine clinical laboratory use. Serious objections are apparent in all the other methods and instruments now employed.

Several years ago I described a method[1] of hemoglobin estimation in which the blood is diluted with N/10 hydrochloric acid in a blood counting pipette and compared with an acid hematin standard in a Hellige colorimeter. In such a method no special pipettes are required, the specimens are taken with the

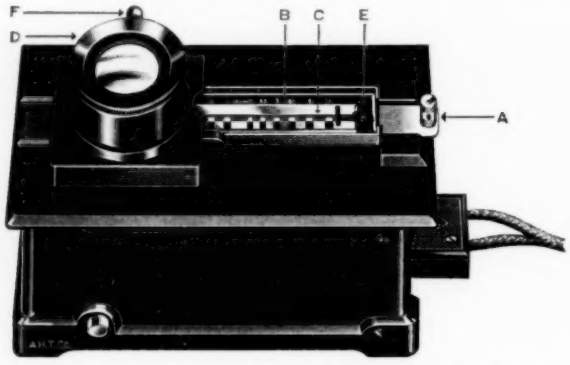

Fig. 1.—Haden-Hausser hemoglobinometer. *A*, slide carrier; *B*, scale in grams; *C*, channel for diluted blood; *D*, reading microscope; *E*, glass standard; *F*, shutter.

routine blood counts, and all readings are made in the laboratory. The method gives satisfactory results but has certain drawbacks. An acid hematin standard suspension settles out and, in time, fades. The cups and other parts of a Hellige colorimeter are fragile. Later I had glass wedges made from the yellow glass described by Newcomer[2] to replace the hollow wedges containing the acid hematin suspension. These were satisfactorily used in the Hellige instrument but the glass wedges are quite expensive to manufacture. C. A. Hausser and Son of Philadelphia, who made one of the wedges for me, suggested the use of a glass comparator slide having on one side a flat piece of colored glass corresponding in color to acid hematin and in the center a channel for the diluted blood corresponding, when covered with a cover glass, to a section of a wedge. Such a slide simplifies both the apparatus and the method. C. A. Hausser and Son have also worked out the other details of the instrument. I have been responsible for the correctness of the calibration of the scale.

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The Haden-Hausser hemoglobinometer can be obtained from the Arthur H. Thomas Company, West Washington Square, Philadelphia, Pa.

DESCRIPTION OF THE INSTRUMENT

The new hemoglobinometer (Fig. 1) consists of a small bakelite case holding a standard 25-watt bulb under a color filter of "daylight glass." A movable carrier (Fig. 2) holding the glass comparator slide (Fig. 3) and cover glass is fitted in a groove on top of the light box, and under a reading microscope. With the slide in place, one sees on looking through the eyepiece a series of illuminated rectangles, separated by alternate dark spaces. The lower half of each rectangle, constituting the color standard complement, is the same throughout the bottom row since the standard is of the same thickness throughout; the upper half of each rectangle, constituting the dilution complement when the channel is filled with diluted blood, is different since the channel has the shape of a wedge, and thus increases in depth from end to end. The figures showing the numerical value in grams of hemoglobin are not visible when the color matching is done but are disclosed by withdrawing a shutter. In making a hemoglobin determination the blood is diluted 1:20 (or 1:10 if the hemoglobin content is below 50 per cent) in a white cell pipette with N/10 hydrochloric

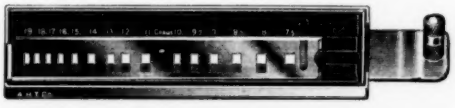

Fig. 2.—Slide carrier with comparator slide in place.

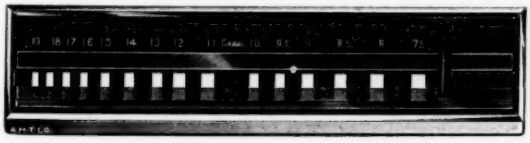

Fig. 3.—Comparator slide.

acid. After standing a few minutes to allow conversion of the hemoglobin into acid hematin the diluted blood is run into the channel of the slide at the end of the cover glass. The channel fills by capillarity. A thin uniform film of the acid hematin will extend also by capillarity from the dilution channel and cover the comparator slide above the color standard. This insures a light transmitting surface common to both dilution channel and standard.

An approximate determination is made by moving the color standard slide to the left until the top illuminated complement (dilution complement) of the rectangle observed in the center of the field closely matches the color standard complement below. The rectangle in which the complements most nearly match is best determined by making such an approximate selection and then contrasting the upper complement with the adjacent color to the left and again with the adjacent color to the right. With the correct rectangle in the center of the field, the dilution complement to the left will be of a lighter tint and the dilution complement to the right of a darker tint, and the dilution and color standard complements of this central rectangle will closely match. Dr. Joseph Razek, of the University of Pennsylvania, has kindly compared the absorption curves of acid hematin and the yellow glass used in the hemoglobinometer. The spectro-

photometric readings have been made with a Razek-Mulder Color Analyzer. The two curves show a close coincidence as indicated in Fig. 4.

CALIBRATION OF THE COLOR STANDARD SLIDE

The color standard slide has been calibrated by the oxygen capacity method using a Van Slyke manometric apparatus,[3] and the Hüfner factor of 1.34 c.c. of oxygen per gram of hemoglobin. The readings are recorded only in grams. A series of dilutions were made from each specimen of blood, the hemoglobin content of which was determined by the oxygen capacity method, using a calibrated 1 c.c. pipette and varying amounts of N/10 hydrochloric acid. The hemoglobin value of each specimen was read by two observers independently. The different dilutions were read at random and not in the order of dilution. The corresponding hemoglobin by the oxygen capacity method was calculated for each dilution. The comparative readings are shown in Table I.

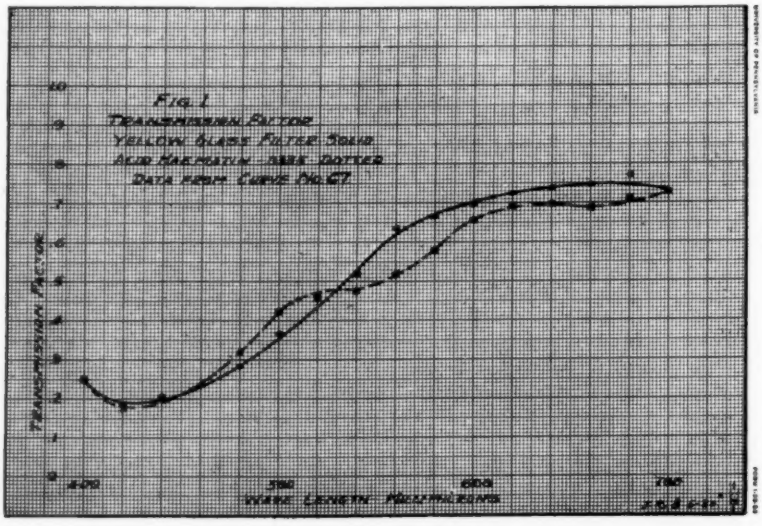

Fig. 4.—Chart showing absorption curves of the standard yellow glass and a solution of acid hematin.

DISCUSSION

The hemoglobinometer described here is a simple, compact, and sturdy instrument. No special pipettes are necessary since standard white cell counting pipettes are employed for the dilution of the blood. The dilution ratio is only 1:20 and can be easily and accurately made. The specimens for the hemoglobin estimation can be taken simultaneously with those obtained for red and white corpuscle counts. The readings can be made conveniently in the laboratory. The instrument is easily cleaned and very little time is required for a complete determination. No fading has been observed of the colored glass so the standard should be permanent. All readings are made in grams, thus allowing the use of any hemoglobin standard desired. The calibration has been made by the oxygen capacity method which is the one of choice for accurate work. Comparative readings with this method and the new hemoglobinometer check closely and different technicians make readings which check closely with each other.

TABLE I

COMPARISON OF HEMOGLOBIN READINGS

SPECIMEN NO.	ACTUAL HEMOGLOBIN CONTENT BY OXYGEN CAPACITY METHOD (VAN SLYKE)	READINGS WITH NEW HEMOGLOBINOMETER	
		OBSERVER NO. 1	OBSERVER NO. 2
	Gm. per 100 c.c.	Gm. per 100 c.c.	Gm. per 100 c.c.
I	14.0	14.0	13.5
	13.3	13.0	13.0
	12.7	13.0	13.0
	12.1	11.5	12.0
	11.6	11.5	11.5
	11.0	11.0	11.0
	10.6	10.5	10.5
	10.2	10.0	10.2
	9.9	10.0	10.0
	9.5	9.5	9.7
	9.2	9.0	9.5
	8.9	9.0	9.0
	8.3	8.5	8.2
	7.8	8.0	8.0
II	15.1	15.0	14.5
	14.4	14.0	14.5
	13.5	13.0	13.0
	12.6	12.5	12.5
	12.0	12.0	12.0
	11.4	11.5	12.0
	10.8	10.5	11.0
	10.3	10.5	10.5
	9.9	10.0	10.0
	9.4	9.5	9.5
	9.0	9.0	9.0
III	13.7	13.5	13.0
	13.0	13.0	13.0
	12.4	12.0	12.5
	11.9	12.0	12.0
	11.3	11.0	11.5
	10.8	10.0	10.5
	10.4	10.0	10.0
	10.0	9.5	9.7
	9.6	10.0	9.5
	9.3	10.0	9.5
	9.0	9.0	8.5
	8.7	8.5	8.2
	8.1	7.8	8.2
	7.6	7.5	8.0
IV	16.9	16.0	16.0
	16.1	16.0	16.0
	15.3	15.0	15.0
	14.6	14.0	14.5
	14.0	13.0	13.2
	13.4	13.0	13.0
	12.9	13.0	13.0
	12.4	12.0	12.0
	12.0	12.0	12.0
	11.5	12.0	11.5
	11.1	11.0	11.0
	10.6	10.5	10.5
	10.1	10.0	10.0
	9.5	10.0	9.5
	9.0	9.0	8.5
	8.5	8.5	8.2
	8.0	8.0	8.0

TABLE I—CONT'D

SPECIMEN NO.	ACTUAL HEMOGLOBIN CONTENT BY OXYGEN CAPACITY METHOD (VAN SLYKE)	READINGS WITH NEW HEMOGLOBINOMETER	
		OBSERVER NO. 1	OBSERVER NO. 2
	Gm. per 100 c.c.	Gm. per 100 c.c.	Gm. per 100 c.c.
V	13.5	14.0	14.0
	12.8	13.0	13.0
	12.2	12.5	12.5
	11.6	12.0	12.0
	11.1	11.0	11.5
	10.7	11.0	11.0
	10.3	10.5	11.0
	9.8	10.0	10.0
	9.4	9.7	9.7
	9.0	9.5	9.5
	8.8	9.5	9.5
	8.5	8.5	9.0

This hemoglobinometer should fill a definite need for a simple and accurate method for hemoglobin determination in the clinical laboratory.

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PRESERVATION OF GROSS SPECIMENS IN NATURAL COLORS*

BY RAYMOND H. GOODALE, M.D., WORCESTER, MASS.

THE preservation of gross specimens removed at autopsy or at operation is an important procedure so that the organs may be used for teaching students or for demonstration at staff conferences. The following method is used in Austria and Germany. I have not seen it described in our text-books and would like to pass it on to others for trial.

Two advantages of this method are: (1) preservation of normal color, (2) the specimen remains practically as soft as when it was removed. The colors have kept well in the museum for as long as two years provided the specimens were not exposed to light except when demonstrated.

PROCEDURE

1. Place organs in Jores solution for two days to two weeks depending on the size. (They may be removed at any time for demonstration provided they are kept moist, but they should be returned to complete the fixation if

*From the Pathological Laboratory, City Hospital, Worcester, Mass.
Received for publication, April 29, 1930.

they are to be used as museum subjects.) Keep the solution in a covered crock in a cool room. Thus the light is kept away from the specimens.

2. Change organs to running water for twenty-four hours, preferably protected from the light.

3. Place organs in preserving solution in jars after arranging for the best exhibition of the pathology. It has been found necessary to add about one per cent of thymol to the preserving solution to prevent the growth of mold.

FORMULAE

Jores Solution:

Chloral hydrate	500 gm.
Sodium chloride	180 "
Sodium bicarbonate	100 "
Sodium sulphate	220 "
Formalin (40 per cent)	500 "
Water	10,000 "

Note: Dissolve chloral hydrate in water before adding other ingredients.

Preserving Solution:

Glycerin	2,000 c.c.
Sodium acetate	800 gm.
Water qsad.	10,000 c.c.

Note: Add one per cent of thymol.

It is advisable to have plenty of Jores solution in excess of the volume of the organs. A wooden cover slightly smaller than the inside diameter of the crock serves two purposes. It keeps the specimens immersed in the solution. It also serves as a surface on which to pin pieces of intestine and blood vessels so that they will fix in the position desired. They are immersed on the under side of the cover. The usual cover is placed on top of the crock to keep out the light.

The Jores solution should be replaced periodically (every three or four weeks), when it smells foul or becomes markedly discolored by blood.

A MODIFIED MELTZER APPARATUS FOR ANESTHESIA IN ANIMALS*†

By H. Livingstone, M.D., and L. S. Hrdina, Chicago, Ill.

A POSITIVE pressure anesthesia apparatus for use in experimental chest surgery is frequently indicated. It is a well-known fact that one of the greatest dangers of intrathoracic operations is the occurrence of pneumothorax as soon as the pleural cavity is opened. This may cause serious respiratory embarrassment or may be fatal. Numerous accounts of this difficulty are found in the medical literature including case reports by Matas[1] and recent experimental chest surgery on dogs by Bettman[2] and Joannides.[3]

The development of devices for artificially inflating the lungs with air dates from ancient times. Hippocrates and Paracelsus (1493-1541),[4] William Hunter, Goodwyn (1788), James Curry (1791), Fine of Geneva (1800), and Cap of Lyons (1828)[4] have reported methods of pulmonary inflation. Finally, Tuffier, Hallion and Quenu[5] demonstrated the value of artificial inflation of the lung through the larynx for the prevention and relief of surgical pneumothorax (1896). Milton,[6] in 1897, operated successfully upon a goat and upon an Egyptian with the use of tracheal insufflation. Doyen,[5] of Rheims, designed the first finished model of an intubating apparatus. Later the Fell-O'Dwyer[1] model appeared. This was modified by Matas[5] so that general anesthesia could be administered with it. In 1898 Parham[7] used the Matas apparatus in the first thoracic operation of record in this country.

Sauerbruch[8] in 1904 developed the negative pressure cabinet which was used successfully on both animals and human beings in the prevention of pneumothorax. Brauer[9] presented a positive pressure apparatus which was modified and improved by Green and Janeway[10] in 1907. This pressure cabinet was also used postoperatively until adhesions had formed between the visceral and parietal pleura.[11] Meyer,[12] in 1910, developed a differential pressure cabinet and later[13] emphasized the necessity of interrupting now and then the pressure, allowing the lungs to collapse. He[14] also advised leaving the patient under differential pressure for some time after the completion of the operation. These differential pressure chambers were very expensive.

Meltzer and Auer[15] perfected and popularized intratracheal insufflation anesthesia. They found that if they passed a tube through the larynx of a dog almost to the bifurcation of the trachea, and blew an air-ether mixture through this tube in a continuous stream, the animal could be kept alive and anesthetized for many hours, even after all voluntary respiratory movements

*From the Department of Surgery, University of Chicago.

†This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Received for publication, April 17, 1930.

had been abolished by curare.[16] In these animals it was possible to open both pleural cavities widely and to have the animals remain alive for many hours. The respiratory tract was efficiently protected from aspiration by the continuous outgoing current of air. Chloroform used in this method led to inflammation of the lungs, but ether proved to be a safe and reliable anesthetic.

Carrel[18] reported very successful experimental operations upon the heart and the aorta using the Meltzer-Auer method of intratracheal insufflation. Elsberg was the first to introduce this type of anesthesia into clinical surgery,[19] after obtaining very satisfactory results in thoracic surgery on dogs.[20] Lilienthal[21] gives a detailed account of the first case of thoracotomy in a human being under anesthesia by intratracheal insufflation. Elsberg[22] devised two forms of the apparatus; one for stationary use, and the other a portable model. Peck[23] used the Elsberg model with fine results on 216 patients. Fischer[24] demonstrated his intratracheal insufflation equipment in 1911, and about the same time Cotton and Boothby[25] reported the use of nitrous oxide and oxygen by intratracheal insufflation. Cotton and Boothby's machine was

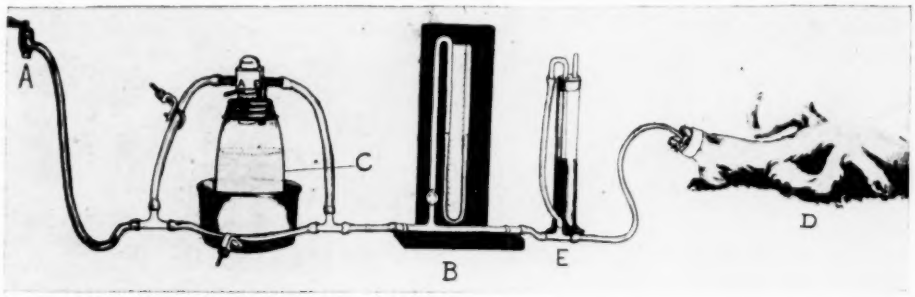

Fig. 1.

an outgrowth of Boothby and Ehrenfried's[26] work with animals. In 1912, Pope[27] constructed an apparatus involving the Auer and Meltzer theory.

Because of its simplicity of construction and the gratifying results it has enabled us to obtain, we are presenting an apparatus which was developed in this institution for use in experimental dog surgery. It is a positive pressure intratracheal insufflation anesthesia apparatus embodying the Meltzer-Auer principle.

APPARATUS

The insufflating apparatus (Fig. 1) delivers an air-ether mixture at a known pressure to the trachea of the dog. Compressed air or an air pump (*A*) may serve as the source of the air current. A manometer (*B*) is placed in the circuit between the ether supply (*C*) and the dog (*D*). A safety device (*E*) is placed between the manometer and the dog. This consists of a graduate cylinder containing mercury, with a connection to the main air-line leading beneath the surface for a depth of 6 or 8 cm. This device prevents the mercury from being forced out of the manometer in case of obstruction to the air flow distal to this point, such as kinking, etc,; thereby raising the pressure.

Another mechanism (Fig. 2) regulates the respiratory outlet. The modified Kunde mask[28] bears a tightly stretched rubber dam containing a perforation for the muzzle of the dog. The other end of the mask is equipped with two metal tube outlets, one bearing the inlet tube which continues into the trachea, and the other, the outlet tube for the air returning from the lungs, around the intratracheal catheter. The outlet tube (*A*) is connected by means of rubber tubing to a graduated metal tube (*B*), which is placed in an 8,000 c.c. bottle of water. An adjustable clamp (*C*) holds the tube in place. Escape of the air-ether mixture around the inlet tube is prevented by means of a clamp (*D*) applied to the loose rubber sleeve (*E*).

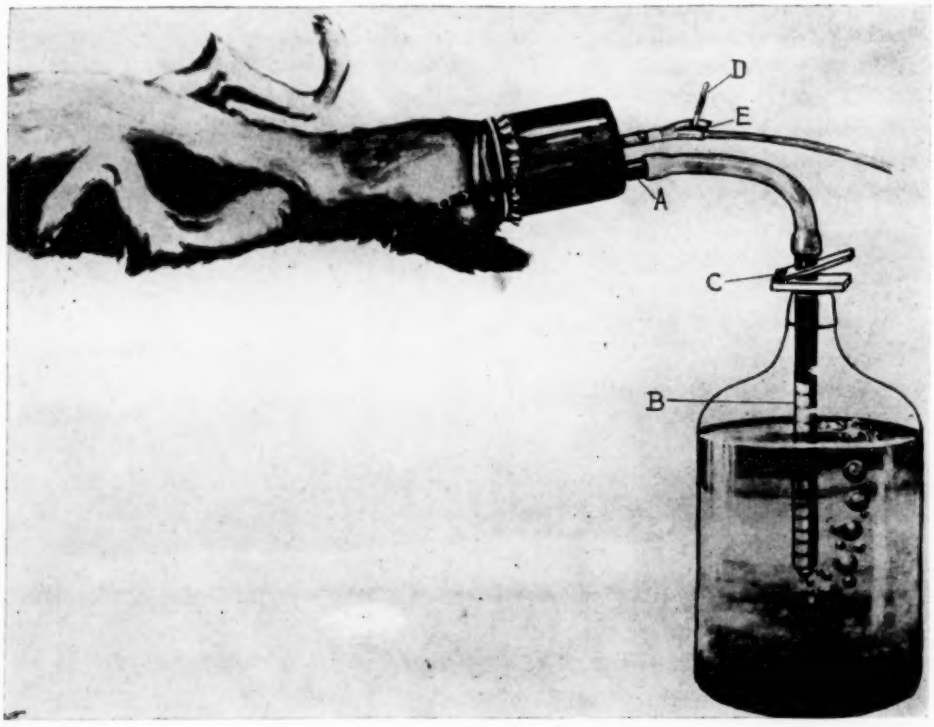

Fig. 2.

The intratracheal catheter used is comparable in size and consistency to a No. 16 French urethral catheter. In dealing with smaller dogs, a smaller intratracheal catheter is indicated.

METHOD

Dogs weighing at least 6 kg. were used. They were given a preoperative dose of morphine gr. ¼ and atropine gr. $\frac{1}{150}$, one-half hour before being placed on the operating table. Anesthesia was induced by means of the tightly fitting mask and ether inhalation apparatus developed in the physiology and pharmacology department of this institution (Fig. 3). This apparatus is a very practical adaptation of an old principle. A 50-50 ether-air mixture was administered.

When the dog was deep enough under anesthesia to abolish the cough reflex, he was placed on his back and his head was drawn to the end of the table and turned about 45 degrees to the left. The operator stood on the

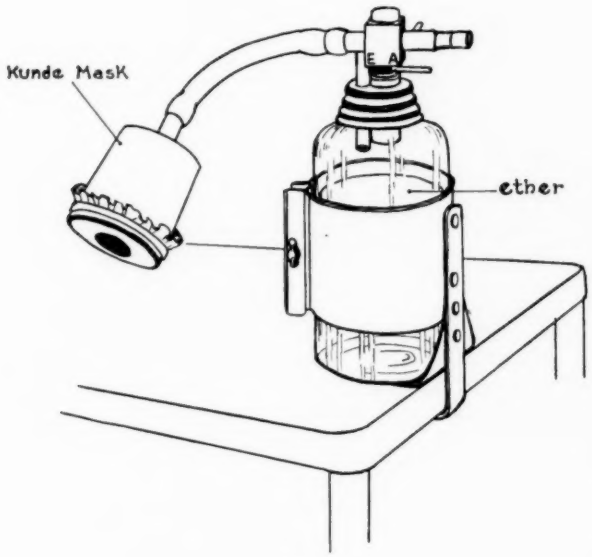

Fig. 3.

Fig. 4.

left side and the assistant on the right. The assistant depressed the upper jaw with his left hand, and with the right hand pulled the tongue forward and upward. The operator then passed his left hand into the mouth, located

the epiglottis, and with the right hand inserted a rubber catheter into the mouth. The left index finger then directed it into the glottis (Fig. 4).

After the glottis was reached, the catheter was inserted into, and passed down the trachea for a distance of 6 or 8 inches. This brought the tip of the catheter to within 1 inch or more of the bifurcation. If air was heard rushing out around the catheter we were sure the catheter was in the trachea. Also, if the stomach did not distend when air was forced through the catheter, we knew that it was not in the esophagus.

The distance from the glottis to the bifurcation of the trachea has been measured in a large series of dogs at autopsy. It appears that this distance varies according to the size of the dog. In dogs weighing 10 kg., the distance is approximately 20 cm. In larger dogs the distance decreases (i.e., 15 kg. = 25 cm.), while in smaller dogs the distance increases (i.e., 5.6 kg. = 15 cm.).

An air-way (Fig. 5) had been previously threaded onto the catheter, and this was now placed so that it held the catheter in correct position in the

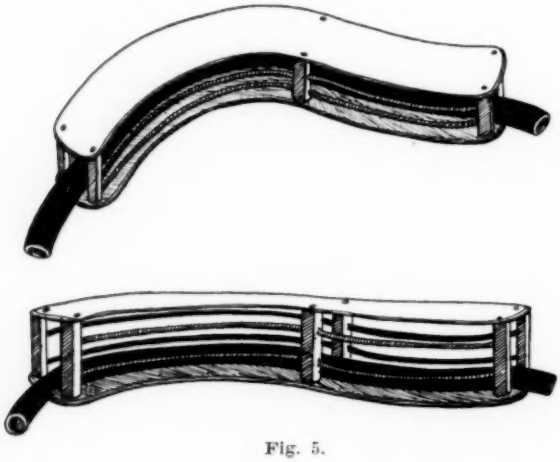

Fig. 5.

mouth, facilitating the free exchange of gases, and also preventing the dog from biting it. The mouth was then closed and a bandage was tied around both jaws and air-way.

The mask (Fig. 2) was run down over the muzzle and strapped into place, making an air-tight approximation. The clamp (*D*) was then applied to the rubber sleeve (*E*) to eliminate leakage of the ether-air mixture expired. The inlet tube was connected with the tube which led from the insufflating apparatus (Fig. 1) and from which the mixture of air and ether was flowing, and the insufflation was begun. The air pressure was maintained at from 2 to 4 cm. of mercury.

The outlet tube of the mask was connected with the mechanism for controlling the respiratory outlet (Fig. 2). The amount of resistance to the outflow of air was determined by the depth of the end of the tube (*B*) under the surface of the water. Thus we determined the amount of inflation at which the lung lobes were kept, the intake being constant.

RESULTS

Positive pressure intratracheal ether anesthesias have been administered during approximately 1,000 operations on dogs, including 900 thoracotomies, 30 cases of cardiac decortication, and 40 cases of bone transplantation to the myocardium; and intratracheal ether anesthesias have been given in 160 cases. Perfect surgical anesthesia was obtained in every instance. Fifty of the above dogs received this type of anesthesia continuously for six hours, and in 2 it was continued for thirteen hours. Twenty of these dogs showed no ill effects following a second and a third operation using the same anesthesia.

COMMENT

In comparison with the various other types of positive pressure anesthesia equipment presented, we feel that this apparatus has many advantages. It is simple and inexpensive in both construction and operation. It is less cumbersome than the various differential cabinets. Our lack of postoperative complications seems to indicate that the elaborate air filtering and ether warming devices on other equipment are unnecessary. However, if the surgeon desires a more delicately controlled anesthesia, using warmed ether vapor, this may be obtained with little difficulty. At this institution all of the above principles have been embodied in a compact apparatus providing for the warming and humidifying of the ether vapor.

The mechanism for regulating the respiratory outlet maintains a constant and safe amount of inflation of the lung lobes. It has not been found necessary to allow the lungs to collapse and be reinflated at frequent intervals. The lungs remain pink and quiet throughout the operation, and the freedom from respiratory movement is very advantageous during thoracic surgical procedures.

Our thanks are extended to Dr. C. M. van Allen for his interest and valuable suggestions in the construction of this apparatus.

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A METHOD OF CENTERING THE CARDIOID DARKFIELD CONDENSER*

By Linda B. Lange, M.D., Baltimore, Md.

GOOD darkfield illumination cannot be obtained without exact centering of the darkfield condenser. In the case of the paraboloid condenser this is simply accomplished by manipulating the condenser by means of its centering screws until the circle engraved on the upper surface of the condenser is centered to the microscopic field as seen with the 16 mm. objective. In the case of the cardioid darkfield condenser this is impossible because no central engraving is present on the upper surface.

Catalogues of optical firms instruct the user of the cardioid condenser to adjust the source of light, the reflecting mirror and the condenser in such a way that the illumination on the slide when the condenser is raised to its lower surface with connecting fluid between the two shall show first, a central round spot of light and, second, an equally bright light reflex from the periphery of the upper circular plane of the condenser. If the light is not reflected from the mirror properly the light intensity as seen at the top of the condenser will show lateral inequalities, and if the condenser is too low the central light area will be in the form of a ring rather than in that of a small evenly illuminated circle. Since these adjustments are all made with the naked eye they are necessarily inexact, and even supplementary attempts

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Received for publication, March 22, 1930.

to adjust the light to reflect equally from all sides of round bubbles as seen through the microscope are rarely entirely successful.

A simple method of achieving perfect alignment of the parts has given satisfaction for a sufficient length of time to assure us of its reliability and justify a communication for the benefit of other users of the cardioid darkfield condenser.

1. Center the condenser in its holder by naked eye observation from below by means of the centering screws or other corresponding centering device.

2. Adjust the source of light so that it will fall on the center of the mirror.

3. Place a drop of connecting fluid on the condenser, place a thin slide with a moderately dense specimen on the stage and raise the condenser to contact with the under surface of the slide.

4. Adjust the light source and the mirror to give a macroscopically correct illumination at the surface of the condenser, i.e., a central spot of light and an even circle of light at the periphery of the top plane of the condenser.

5. Focus onto the slide using the 16 mm. objective.

6. Lower the condenser until a dark circular area appears in the field. (It is at this point that a moderate amount of reflecting material in the specimen is helpful.) When the gross adjustment (1) has been made carefully, the dark spot will fall within the low power microscopic field. The dark area is due to the lowering of the focal point of the condenser to a plane below that of the specimen, and section by the plane of the slide at the level of the specimen of the dark cone within the peripheral "pencil" of light above the focus or crossing of the light rays. Center the condenser to bring the dark area to the center of the low power microscopic field. Raise the condenser to focus the light at the plane of the specimen.

Review the adjustment of the light source and mirror so that round bubbles in the center of the microscopic field which are undisturbed by light diffusion from peripheral objects will give a symmetrical reflection of light. Repeat step 6.

When the reflex from a bubble in the center of the microscopic field is symmetrical and the round dark area produced by lowering the condenser falls in the center of the low power microscopic field the optical system is in alignment and the best results are obtained with the cardioid darkfield condenser.

It is our custom to use distilled water as a connecting medium between condenser and slide. While slightly less efficient than immersion oil for this purpose, the convenience is so much greater that its advantages far outweigh its disadvantages for routine darkfield work.

THE NORMAL BLOOD COUNT OF THE RABBIT*

By J. Warren Jackson, M.D., and W. D. Stovall, M.D., Madison, Wisc.

CLINICAL investigation often necessitates the use of laboratory animals. The normal or control animal is, of course, necessary in such investigations. In studying the effect of any agent on the formed elements of the peripheral blood stream, such controls are essential. There appears to be a variety of opinions as to the constancy of the blood counts of rabbits. While attempting to establish such figures preliminary to another problem, we have secured some interesting results as to the constancy of the red blood counts and the daily variation of the white blood counts of rabbits.

Jordan and Kindred[1] in their textbook of *Embryology* establish the red blood count as varying from 6,300,000 to 7,200,000 per c.mm.

Orr[2] in a comparison of blood counts before and after splenectomy used rabbits with white counts varying from 5,200 to 17,025 cells per c.mm. He notes one red blood count of 5,680,000 cells per c.mm.

Weiskotten, Gibbs, Boggs and Templeton[3] found the average white blood count to be 11,892 cells per c.mm. from counting five rabbits with an average of five counts on each rabbit. They found the average red blood count to be 6,126,750 cells per c.mm. on five rabbits.

Bushnell and Bangs[4] in a series of daily counts found a red blood count of 6,632,000 cells per c.mm. at 10:15 and at 1:15 found a red count of 4,568,000 cells per c.mm. On the same rabbit six days later at 8:45 the red blood count was 6,976,000 and at 1:15 was 4,616,000 cells per c.mm. They give the extreme limits on a series of daily and hourly counts from 4,322,000 to 6,976,000 cells per c.mm. and give the mean of the total erythrocyte count as 6,600,000 and give the average deviation from the mean as 790,000 cells per c.mm. They give 10,200 to 13,600 cells per c.mm. as the extreme limits of the total white blood count. The total leucocyte count is approximately 12,500 and the average deviation from the mean is 800. They found no parallel between the number of total leucocytes and the percentage values of small lymphocytes and polymorphonuclear leucocytes. Bushnell and Bangs give the average proportion of white blood count as: Polymorphonuclear leucocyte count as 39.10, the small lymphocytes as 53.90, large lymphocytes as 2.55, large mononuclears as 0.43, eosinophiles as 1.12, basophiles 3.58, transitional cells 1.07.

R. H. Mole[5] gives the red blood count as varying from 4,670,625 to 7,818,750 with an average of 6,567,609 cells per c.mm. He gives the total white blood count average 10,232 cells per c.mm. with 16,250 maximum and minimum 5,625.

Gulland and Goodall[6] give the extreme limits as 5,760,000 to 4,560,000 red cells per c.mm., the total white count as 7,200 to 16,000 per c.mm.; poly-

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Received for publication, January 27, 1930.

morphonuclear leucocytes 32 to 50 per cent; lymphocytes 48 to 62 per cent; eosinophiles 1 to 48 per cent; basophiles 1 to 2 per cent; hemoglobin 70 to 90 per cent.

In view of these findings, we undertook to determine:

1. The degree of variation in a given day.
2. Variations from day to day over several weeks.
3. The relationship between the total leucocyte count and the differential count.

In order to obtain the variation during the day, counts were made at 9 and 11 A.M and 2 and 4 P.M. The influence of feeding upon the counts was observed by a study of blood taken before and after the animals were fed.

METHOD OF PROCEDURE

Each rabbit was kept in a separate pen. They were fed at 9 A.M. and at 4 P.M. All rabbits had access to water at all times. Blood was obtained from a cut across a small venule of the ear and diluted in Trenner automatic pipettes. The cells were counted in Levy counting chamber with improved Neubauer ruling. Both pipettes and counting chamber were certified by the United States Bureau of Standards. Both sides of the double counting chamber were filled and the counts averaged for all results. The Tallqvist scale was used to estimate the hemoglobin.

The blood smears were made by the Ehrlich cover slip method and stained with Wright's stain. One or two hundred cells were counted and the cells classified as lymphocytes, polymorphonuclear leucocytes, endothelial cells, eosinophiles and basophiles.

TABLE I

COMPLETE BLOOD COUNTS ON FOUR RABBITS OVER A PERIOD OF TIME VARYING FROM FIVE DAYS TO ONE HUNDRED AND TWELVE DAYS

	NO. OF COUNTS	NO. OF DAYS	R. B. C.*	HB.	W. B. C.	POLY.	LYMPH.	ENDO.	EOS.	BASO.
Rabbit No. 27—Male										
	11	40								
Maximum			5.440	90	12,050	66	28	3	3	4
Minimum			4.730	85	8,550	27	68	0	0	0
Average			5.055	85	10,713	37.2	60	1.4	1.2	1.8
Rabbit No. 26—Male										
	9	62								
Maximum			5.700	90	10,750	39	73	4	7	7
Minimum			4.000	85	5,000	23	49	1	0	0
Average			5.008	85	8,352	31	62	2.2	1.5	2.7
Rabbit No. 35—Male										
	2	5								
Maximum			5.145	90	12,250	45	58	2	2	4
Minimum			4.975	90	10,950	34	50	1	1	3
Average			5.060	90	11,600	39.5	54	1.5	1.5	3.5
Rabbit No. 29—Male										
	5	112								
Maximum			5.515	90	13,000	43	62	2	4	5
Minimum			4.650	75	9,300	32	49	1	0	2
Average			5.057	85	10,770	38.4	54.8	1.6	1.4	4.2
Average of 27 counts			5.040	85	10,416	31.8	58.6	1.8	1.84	2.73

*Red counts in millions per c.mm.

DISCUSSION

The counts tabulated in Table I show the maximum and minimum variation of complete counts over a varying period of time. These counts were done at various hours during the day. The average count of each rabbit is given and the maximum and minimum counts over the period of time indicated in the table. The average for the group of twenty-seven counts is given at the bottom of the table.

Table II shows the variations of the individual counts of fifteen rabbits without any reference to time of day or intake of food. Rabbits Nos. 4 and 12

TABLE II

SINGLE BLOOD COUNTS ON FIFTEEN RABBITS

RABBIT NO.	SEX	R. B. C.*	W. B. C.	DIFFERENTIAL COUNT					
				NUETRO.	LYMPHO.	ENDOTHEL.	EOSINOPHILES	BASO.	HEMOGLOBIN
1	M	5.710	10,450	44	49	3	2	2	85
2	M	5.820	11,650	23	65	6	2	4	85
3	M	6.030	12,200	30	57	6	3	3	85
4	M	4.510	12,650	40	52	4	1	3	85
5	M	5.090	9,900	67	27	1	2	3	85
6	M	4.920	12,350	54	37	2	2	5	80
7	M	5.460	13,000	40	52	2	2	4	85
8	F	4.740	8,450	45	46	5	2	2	80
9	F	4.900	9,200	52	38	4	1	3	85
10	M	5.870	11,850	39	52	6	2	1	80
11	F	5.220	14,750	47	43	7	0	3	80
12	M	6.600	10,100	43	44	5	3	5	80
13	F	5.140	11,375	47	45	5	1	2	85
14	F	5.230	8,900	51	43	4	1	1	80
15	F	5.000	9,350	42	47	5	2	4	75
Average		5.308	10,878	44.2	46.4	5.0	1.7	3.0	

*Red counts in millions per c.mm.

show a marked variation in red blood count, but the group average, 5,308,000, is very close to the average, 5,040,000, given in Table I.

The total white counts of Tables I and II are very close, but the differential counts show a wide variation. The counts recorded in Table I are the results of separate counts on four rabbits over a period of weeks, while the counts recorded in Table II are the result of single counts on fifteen rabbits from the stock pens.

Table III records the result of the daily variation of W.B.C. and R.B.C. counts on three rabbits done over a period of three weeks. A study of this table also indicates a very close agreement in total counts with the results recorded in Tables I and II.

Fig. 1 is drawn from the total W.B.C. counts recorded in Table III, and differential counts done at the same time. This illustrates graphically the change in total and differential counts during the day. The percentage of lymphocytes and polymorphonuclear leucocytes were converted into terms of absolute counts in order to facilitate the plotting of a curve. The nine o'clock counts were done before the morning feeding and the four o'clock counts before the afternoon feeding. The graph shows a sharp rise in total white count at eleven and two o'clock, and at four o'clock it is back to the

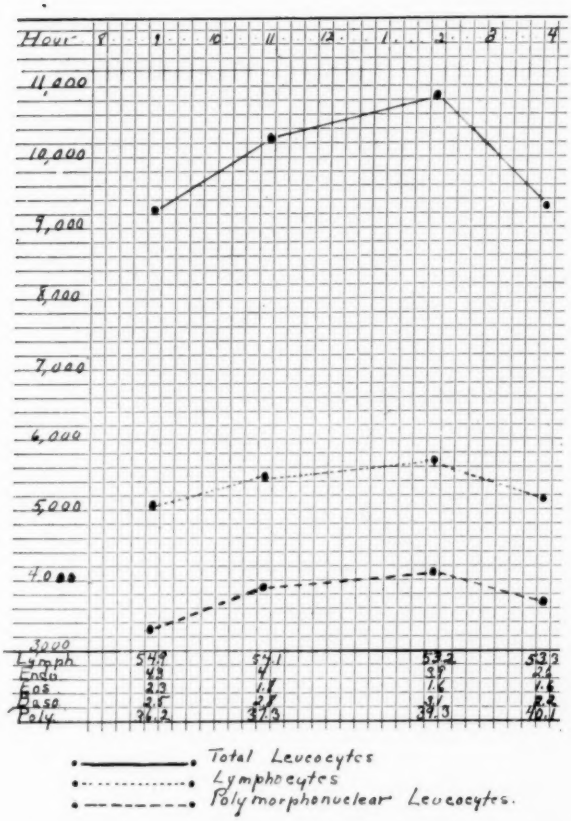

Fig. 1.—The relation of the daily variation of total white count and differential count.

TABLE III

VARIATION OF BLOOD COUNTS DURING THE DAY

RABBIT NUMBER	DATE YEAR: 1929 MO.	DAY	TOTAL W. B. C. A.M. 9	A.M. 11	P.M. 2	P.M. 4	TOTAL R. B. C.* A.M. 9	A.M. 11	P.M. 2	P.M. 4
31	7	22	9,300	10,650	10,600	9,250	5.340	5.450	5.570	5.310
31	7	30	8,700	10,150	10,320	9,050	5.730	5.650	5.330	5.700
31	8	9	5,450	7,300	7,700	6,200	5.850	6.020	5.890	6.100
31	8	13	8,750	9,400	10,100	8,750	5.750	5.530	5.580	5.610
32	7	23	8,850	10,050	9,980	8,950	5.270	5.000	5.330	5.260
32	7	30	8,200	11,000	9,750	8,400	5.680	5.740	5.460	5.520
32	8	7	9,950	13,000	10,370	9,800	5.790	6.000	5.630	5.800
32	8	12	7,425	8,750	8,450	7,450	5.740	5.780	5.630	5.580
32	8	14	11,200	11,400	12,100	10,950	5.340	5.610	5.280	5.450
33	7	23	12,320	12,325	13,135	12,550	5.135	5.115	5.220	5.260
33	8	2	12,020	13,430	13,200	12,130	5.340	5.620	5.230	5.500
33	8	9	12,150	13,200	12,750	9,915	5.030	5.340	5.310	5.420
33	8	13	10,250	11,100	11,600	11,000	5.630	5.710	5.680	5.060
Average			9,328	10,319	10,755	9,352	5.509	5.582	5.472	5.505

Average total R.B.C. count 5,517,000
Average total W.B.C. count 10,207

*Red blood counts in millions per c.mm.

nine o'clock level. The lymphocyte and polymorphonuclear leucocyte counts show a parallel increase and decrease during the four periods of counting. The endothelial cells, eosinophiles and basophiles show no significant change and the average given in Fig. 1 is in per cent.

We do not have sufficient counts to make any definite conclusions, but suggest that digestion may account for an increase of the total leucocyte count for a period of four or five hours following intake of food.

A study of Table III reveals a constant R.B.C. count throughout the day. The maximum average count is 5,582,000 cells per c.mm. (11 o'clock), and the minimum average count 5,472,000 cells per c.mm. (2 o'clock), or a variation of only 110,000 cells.

The greatest variation in the total red blood count of any rabbit for any one day is in the case of Rabbit No. 31 under date of July 30, where there is a maximum count of 5,730,000 and a minimum count of 5,330,000, or a difference of 400,000 cells per c.mm.

SUMMARY

The rabbits we have studied have shown constantly a daily variation of the W.B.C. count. This variation is a rising count after the nine o'clock morning count until a peak is reached at two o'clock, when it again declines to the level of the first count in the morning. The tables and chart give the maximum and minimum counts for the day. We have suggested that the intake of food may explain this variation. An average of ninety-four total white counts gives us a count of 10,371 cells per c.mm. The variation from this average is determined by the time of day the count is made and by individual rabbits. Occasionally a rabbit regularly gives a low count, five to six thousand. Infrequently a rabbit will be found which maintains regularly a high count, twelve thousand or above. It is seen, however, that our rabbits maintained counts regularly very close to the average.

A comparison of our total white counts and differential counts shows that the polymorphonuclear leucocytes and lymphocytes play about an equal part in this daily variation.

From seventy-four differential counts we found an average of 37.3 per cent polymorphonuclear leucocytes, 54.1 per cent lymphocytes, 3.1 per cent endothelial leucocytes, 1.81 per cent eosinophilic leucocytes, and 2.89 per cent basophilic leucocytes.

A study of the total red cell count on three rabbits over a period of three weeks each has shown no daily variation. We have found the average total red cell count after a study of twenty-two rabbits (ninety-four counts) to be 5,346,000 cells per c.mm.

In this study the hemoglobin percentage has ranged around eighty-five using the Tallqvist scale.

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A NEW APPARATUS FOR ADMINISTERING VOLATILE ANESTHETICS INTRATRACHEALLY WITHOUT TRACHEOTOMY*

By Lester J. Sawyer, Berkeley, Calif.

MANY operators have experienced difficulty with anesthetics administered to small laboratory animals by means of various cone inhalation devices. Ether and chloroform cause irritation of the mucous membrane of the respiratory tract, and thus induce reflex secretion of saliva and mucus. The mechanical obstruction of the upper air passage set up by the accumulation of these fluids, or the falling back of the tongue into the throat is often responsible for embarrassed respiration and consequent death to the animal. It is because of these difficulties that an urgent need was felt for a simple, non-failing respiratory apparatus which can be used for intratracheal anesthesia without tracheotomy.

It is of the greatest importance in administering a volatile anesthetic that the vapors shall be diluted with air. In animal experiments it has long been established that in this way it is possible to obtain a safe anesthesia of many hours duration, and that a far lower concentration is required for the maintenance of the anesthesia than for its induction. The goal to be constantly striven for is an anesthesia with air which contains the anesthetic vapors in the minimal efficient concentration. The apparatus about to be described fulfills the desired requirements and overcomes the usual difficulties.

Fig. 1 gives a diagrammatic representation of the apparatus. Sulfuric acid (C.P.) is placed into bulbs *2* and *3* to a level about one-eighth of an inch above the lower ends of the tubes *X* and *Y*. Bulb *1* is half filled with ether or any volatile anesthetic to be used by opening stopcock *M*. The drain cocks *A*, *B* and *C* need not be opened except in the event that it is desired to change the liquids in the bulbs. Tube *T*, a slightly curved metal or hard rubber cannula of appropriate size and attached to a short length of rubber tubing, is inserted into the trachea of the animal. Upon inspiration, if stopcock *M* is entirely open, air will be drawn through *Z*, bubble up through the ether in bulb *1* and pass through *O* carrying with it ether vapor into the lungs. No air will enter through *Y* since, due to the difference in specific gravity between ether and sulfuric acid, less resistance is offered to the passage of air by the ether column. On the other hand, if resistance is introduced by slight

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Received for publication, March 25, 1930.

or complete closure of stopcock *M*, air will be drawn through *Y* causing inspiration of air and ether, or air only. In this way the concentration of air and ether can be controlled through a single adjustment, viz., stopcock *M*. The air-ether mixture is drawn through the main line O from bulbs *1* and *2* but the sulfuric acid in bulb *3* makes it impossible for air to enter tube *X*. Now on expiration the gases travel through tube *X*, bubble through the sulfuric acid in bulb *3* and out through the opening *N*, it being impossible to escape through tubes *Y* and *Z*, due to the resistance offered by the columns of sulfuric acid and ether respectively. The cycle is repeated continuously so long as respiration goes on.

In this way a simple valve system is operated by the normal respiratory processes of the organism without involving external pumps or mechanical valves. Failure of the valve mechanism is obviously almost impossible. One stopcock regulates the entire apparatus. The air-ether mixture can be so adjusted that the desired intensity of anesthesia can be maintained during the entire operation without further attention. Little resistance is offered to

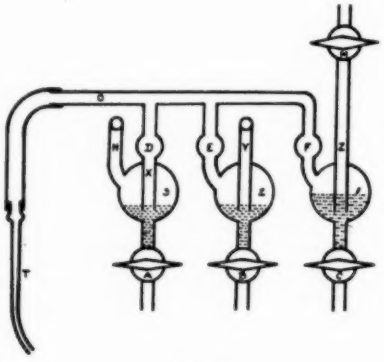

Fig. 1.

free respiration. Atmospheric pressure is maintained throughout the apparatus. The sulfuric acid affords clean dry air and remotely possible fumes are guarded against by bulbs *D*, *E* and *F*. Small amounts of carbon dioxide are always mixed with the inspired ether and air, thus acting advantageously as a respiratory stimulant. Very small amounts of the anesthetic are required because of the extremely high efficiency of the apparatus, thus making it ideal for administering limited amounts of experimental anesthetics.

Animals have been anesthetized repeatedly and for prolonged periods with no apparent injuries other than the usual ones inherent in the anesthetic itself.

There are no troublesome cones interfering with head and neck operations. The irritation of the oral cavity has been reduced to a minimum.

The apparatus can be made to scale in accordance with the size and respiratory requirements of the animal. One-fourth inch glass tubing and one inch bulbs have been found convenient for rats and animals of similar size.

With practice the tube *T* can be inserted from the very start into the trachea with little or no discomfort to the animal. Slight anesthesia may be

induced prior to insertion of the tracheal cannula in order that no injury will result in a very restless animal during the insertion.

It is the opinion of the writer that the employment of the apparatus here described will decrease the number of fatalities of laboratory animals and will eliminate much of the inconvenience heretofore experienced by operators in administering anesthetics.

A NEW HIGH EFFICIENCY GLASS CONDENSER*

By Paul R. Crandall, Battle Creek, Mich.

THERE are several more or less standard types of glass condensers available for general laboratory use. They may all be listed, however, in one of two classes: (1) those in which the cooling medium forms a jacket surrounding the distillate, and (2) those in which the vapors to be condensed pass over tubes or coils carrying the cooling agent. Either type is satisfactory when the required capacity is small, but in large sizes they are cumbersome and inefficient. When a large volume of liquid is to be distilled, the time consumed is undesirably long unless several stills are set up to operate simultaneously. This also has its disadvantages.

Such a situation is encountered in the preparation of colloidal gold solution. For this purpose water, *freshly re-distilled* through an all glass system, is prerequisite. When several liters of gold solution are to be made up, this means the production of a large quantity of distilled water in a relatively short time.

Formerly, in this laboratory, four 30-inch straight Liebig condensers were used. Such a set-up uses considerable desk space and requires more or less constant attention. It seemed, therefore, that a single unit would be preferable, if it could be made compact enough to be easily handled and at the same time of sufficiently large capacity.

The condenser† here described and illustrated was designed to meet these requirements. It is, in effect, a combination of the two types mentioned above. The details of construction can be seen from Fig. 1. The cooling water enters at "*a*," fills the outside jacket and overflows at "*b*" into the inner tube which is bent into a double "U" so that the water traverses the steam column three times. This gives quite as much cooling surface as would a coil and is easier of construction. The cooling water is finally discharged at "*c*." No difficulty is encountered in keeping a solid water column in the downward flowing tubes, once they are completely filled. A little excess pressure when the water is first turned on readily starts the syphons, after which the volume of flow may be decreased. Since there is a positive flow through

*From the laboratory department of the Battle Creek Sanitarium, Battle Creek, Mich.

†This condenser was made for us by Will Corporation, Rochester, New York. Units may be obtained from them built to the author's specifications.

Received for publication, April 4, 1930.

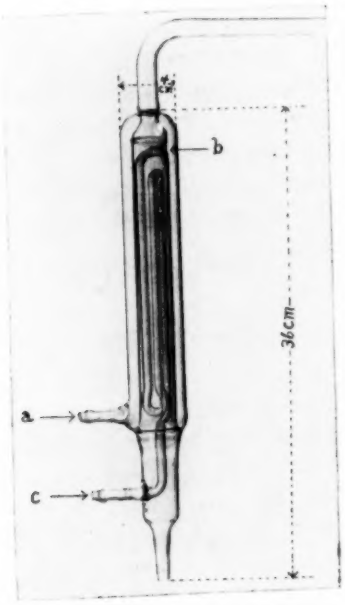

Fig. 1.

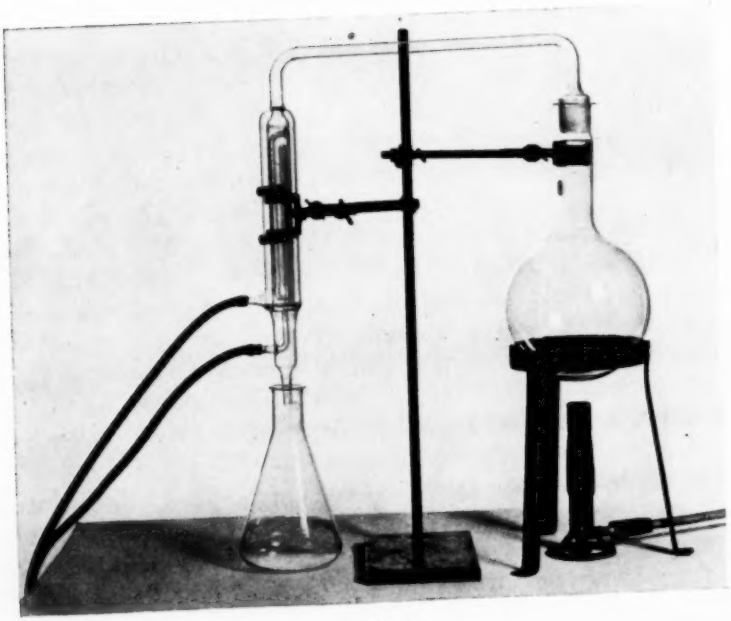

Fig. 2.

the system there is small chance of eddy currents and cooling is very complete. The volume of water required is therefore relatively small, as it may be discharged only a few degrees lower in temperature than the distillate. One unit of the size indicated will produce in excess of two liters of distilled water per hour, which is about the maximum obtainable from one Meeker or Fisher burner as the source of heat. The over all space used for the set-up is small and it requires a minimum of attention. If two boiling flasks are available, one may be heated while the other is distilling and then interchanged, so as to keep the condenser in continuous operation.

The apparatus should prove of value also for the distillation of highly volatile solvents, its large cooling surface insuring complete condensation. In a small size it becomes a very efficient and easily handled reflux. It is short and light in weight, since the volume of cooling water retained is small. Also there is no chance of trapping the condensate to produce "priming."

The glass used is Pyrex and for the specific purpose mentioned, i.e., the production of distilled water for colloidal gold, where an all glass set-up is necessary, connection is made to the boiling flask by a ground joint as shown in Fig. 2. For general use the condenser may be obtained with the ordinary straight neck and in size to meet any requirement. Soft glass might be used but is not recommended on account of the several seals required in its construction. The greater mechanical rigidity of Pyrex offsets its slight difference in cost.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUBERCULOSIS: The Leukocytic Reaction, Dunlop, D. M. Edinburgh M. J. 37: 174, 1930.

From a study of 500 cases the author concludes:

The blood picture in tuberculosis, even in very chronic cases, differs from that seen in health, and this difference is not entirely due to secondary infection.

In health the ratio between the neutrophiles and lymphocytes is less than the ratio between the lymphocytes and monocytes. This picture is reversed in tuberculosis, and the more it deviates from the normal the worse the prognosis.

If the neutrophiles be taken as an expression of activity, the lymphocytes of resistance or healing, and the monocytes in certain definite circumstances of new tubercle formation and in others of healing, then, by following the leucocytic picture week by week, it is possible that a very accurate index of the progress of the disease may be obtained and of the patient's reactions to it.

If such claims are justified we have at our disposal a prognostic agent of great value and subtlety, which is not made less useful by the fact that it may be performed in a few minutes by any doctor in his consulting room.

STREPTOCOCCI: Identification of Hemolytic Streptococci in Milk, Hardenbergh, H. G. N. E. Med. J. 202: 373, 1930.

Milk to be examined may be either market milk, mixed herd milk, composite samples from groups of cows, or samples from individual cows, or even from individual quarters of the udder of a single cow. Instead of nutrient agar, the milk is plated in blood agar, made by adding about five per cent of defibrinated horse blood to veal infusion agar or to nutrient agar (Difco). In the case of samples from individual cows or quarters of an udder the dilution employed is 1 to 100. In the case of all composite samples, a dilution not greater than 1 to 20 should be used in order to lessen as much as possible the ultimate dilution of any single cow's product that might contain the hemolytic streptococci for which search is being made.

Plating.—One cubic centimeter of the diluted milk is placed in Petri dishes, about 10 c.c. of freshly prepared blood agar is added and mixed, and the plates incubated for eighteen to forty-eight hours. At the end of this time, the plates are examined for colonies showing a zone of hemolysis about them. If typical colonies of the beta type are found, they are selected for further study.

Suspected Colonies.—On the basis of hemolysis in blood-agar plates, streptococci may be roughly divided into two groups, alpha and beta. The former are recognized by a narrow greenish or greenish gray discoloration immediately about the colony, this zone being due to a partial dissolution of the red blood cells. Such streptococci are not known to have any significance to human health. The beta type of hemolytic streptococci exhibit a fairly wide zone (2 to 3 mm. diameter) immediately about the colony in which there appear to be no red blood cells left.

Streptococcus epidemicus produces colonies of the beta type and the surface colonies are especially characteristic, being large, moist and spreading, and seeming to disintegrate when touched with a platinum needle. The deep colonies are large and lenticular in shape. When suspected types of this sort are found, they are to be subjected to specific identification tests as developed by Brown, Frost, and Shaw.

Hemolysis Test.—Characteristic colonies are fished into serum broth, made by adding

a few drops of sterile horse or cow blood serum to previously tubed and sterilized nutrient broth. After twenty-four hours' incubation, the resulting growth is tested for hemolytic power by the tube method. One-half c.c. of the serum broth culture is mixed with a like quantity of a 5 per cent suspension of washed rabbit or horse blood corpuscles in physiologic salt solution. The mixture is placed in a water-bath or incubator at 37° C. for two hours. If the resulting hemolysis is negative or only slight, the culture is discarded as not being S. epidemicus. If the hemolysis is moderately or strongly positive, the culture is held for further tests, and inoculated into dextrose serum broth (for P_H determination), into sodium hippurate broth (for hydrolysis of sodium hippurate test), and upon a blood-agar slant (for capsule examination).

P_H Determination.—Cultures are grown in dextrose broth, to which serum has been added, and incubated for forty-eight hours. The final hydrogen-ion concentration is then determined by the colorimetric method and noted. If the P_H is 4.5 or 4.6 the cultures may be discarded as not characteristic of S. epidemicus, which has a P_H of 4.8 or above.

Sodium Hippurate Test.—Suspected cultures are grown in broth of the following composition:

10 gm. peptone (P. D. & C.)
5 gm. pepsin
0.03 gm. calcium chloride
10 gm. sodium hippurate (Eastman Kodak Co.)
1 drop 1 per cent ferric chloride
1000 c.c. distilled water

Dissolve all the ingredients in the distilled water, adjust to P_H of 7.1 with normal NaOH solution, tube in 5 c.c. quantities, and sterilize in the autoclave.

Cultures are grown in this broth for forty-eight hours or longer. Ferric chloride (7 per cent solution) is then used to test for hydrolysis of the sodium hippurate, 0.5 c.c. being added to exactly 2 c.c. of the broth culture, thoroughly shaken and allowed to stand for a few minutes. If the sodium hippurate has been split, a heavy precipitate is formed (and the culture under test is not S. epidemicus); if the sodium hippurate has not been split or hydrolyzed, the mixture becomes clear (and the culture may be S. epidemicus).

Capsule Determination.—Certain types of pathogenic streptococci and some nonpathogenic types are possessed of a capsule. Since S. epidemicus is encapsulated, it is well to examine suspected cultures for this item. Cultures that have been grown on blood-agar slants for twenty-four hours are suitable. Some of the growth is removed with a platinum loop or needle, mixed with India ink on a slide and examined under the microscope. The presence of a capsule is indicated by a clear zone about the individual cocci that are visible in the India ink preparation. Care must be exercised, however, not to be misled in this test by an unsatisfactory ink as it frequently happens that India ink itself may be contaminated by organisms which show capsules that may be confused with S. epidemicus.

Sugar Reaction.—If any or all of the above tests give results which confirm the suspicion of S. epidemicus being present, inoculations are made into serum broths containing lactose, saccharose, mannite and salicin. These sugar broth cultures are incubated for five days and then tested for acid production. S. epidemicus ferments lactose, saccharose and salicin, but does not ferment mannite.

Pathogenicity Tests.—If it is desired to test further the specificity of any culture identified by the above means, animal inoculation may be resorted to. S. epidemicus is pathogenic for white mice and kills them in seven to forty-eight hours after intraperitoneal injection of one-half c.c. of a twenty-four-hour serum broth culture.

GASTRIC ANALYSIS: The Interpretation of, Anderson, A. F. R. New York State J. Med. **29**: 1497, 1929.

The method followed by the author is as follows:

1. The patient is given the usual duration meal of one-half cup of half-cooked rice and one dozen raw raisins eight or ten hours before applying for the test, and nothing to eat or drink thereafter.

2. On morning of test, the Rehfuss tube is immediately introduced into the stomach and all fasting contents are removed and measured.

3. The stomach is lavaged two or three times, or until clear by having the patient drink three or four ounces of water alongside the tube and removing it by suction through the tube. The stomach can thus be demonstrated to be empty.

4. With the tube in situ, the patient is given 300 c.c. of water to drink, and during the swallowing of this water is given 0.3 c.c. of a 1:1000 solution of histamine dihydrochloride (Imido, Roche) hypodermatically. By the old method powdered cracker was added to the water, its place now being taken by the histamine, which begins to induce secretion at about the time of the first fifteen-minute interval and therefore corresponds closely to the cracker and water meal.

5. At fifteen-minute intervals after the taking of the water meal and histamine, specimens of gastric contents, 4 to 6 c.c. at a time, are removed and placed in a row of bottles, being filtered if their consistency is too thick.

6. At the one and one-half hour point, the stomach is completely emptied through the tube, the residue obtained being measured. This emptying is not to be done unless one or two of the later specimens have been stained with bile. This often does not occur for from two to three hours. If no duodenal regurgitation occurs over a longer period, the examination will have to be terminated. Such delay is due either to pylorospasm or to pyloric stenosis.

7. With the patient lying down and the abdomen exposed, the stomach is inflated through the tube, the size and shape of the stomach and the location of the greater curvature being noted.

8. The tube is withdrawn and the patient is given a drink of water and a short rest before receiving further instructions in regard to his gastrointestinal study.

The examination of the specimens obtained is done in accordance with the following routine and all the findings are immediately entered upon a chart which becomes a part of the patient's record:

1. The first or duration specimen, having been measured and titrated, is examined microscopically for rice and raisin residue, gross blood or pus or other admixtures. A slide specimen, stained with Lugol's solution, is then examined microscopically for starch granules, raisin fragments, other food remnants, yeasts, sarcinae, lactic acid bacilli, blood, pus, giant cells, carcinoma cells, parasites, or other cytologic evidences of disease. In the absence of free hydrochloric acid, a few drops of a filtered specimen are dropped into a very dilute ferric chloride solution to test for lactic acid. The information to be obtained from this single fasting specimen is often of more value than that obtained from all of the remaining specimens, and the examination should be performed without delay and with the greatest possible care.

2. All specimens, including the fasting specimen, are examined for free hydrochloric acid and total acidity, 1 c.c. of the contents being titrated against 1/100 normal sodium hydroxide solution, dimethylamidoazobenzol and phenolphthalein being used as indicators. The acid values therefore are the same as when using 10 c.c. of contents and 1/10 normal solution. These acid values, being charted as tested, produce curves of acidity for diagnostic study.

3. Bile, mucus, pus, and other gross admixtures may be noted from their color and microscopic appearance.

4. Blood, if visible, is charted as $^{v}+^{o}$, if occult $+^{o}$. Occult blood is tested for as follows: A few grains of two parts (by volume) of benzidin and one part of barium dioxide, is rubbed up in a Petri dish with enough glacial acetic acid to produce a thin, smooth smear on the bottom of the dish. A drop of each specimen to be tested is then dropped around the outer edge of the mixture, a deep bluish black color at the margin of contact between the specimen and the reagent being evidence of the presence of occult blood. Eight or ten specimens may be examined with one preparation.

5. Formerly a Wolff-Junghans test was performed on each specimen and a curve produced, which, however, was found to be of doubtful significance. The test was therefore abandoned.

INTESTINAL BACTERIA: Three Methods of Obtaining Intestinal Material . . . Without Contamination, Paulson, M. Am. J. Med. Sc. **179:** 3, 1930.

1. The first device consists of 7 to 8 mm. soft laboratory glass tubing in three lengths: 8 cm., 17 cm., and 30 cm. Freely movable within the glass tube is a cotton swab attached distally to the roughened end of a firm iron wire applicator of 16 gauge. The iron wire applicator is about 12 cm. longer than the length of the glass tubing which encloses it. The increase in length of the iron wire over that of the tubing is the length of that part of the applicator which extends proximally beyond the tubing. This extension is used for two purposes: (1) to make a small handle and (2) to permit projection of the applicator for about 8 cm. beyond the distal portion of the glass tube when the device is in use.

In further preparation, a small pledget of raw cotton is placed at each of the two ends of the glass tube so as to keep the applicator from slipping out of the distal end and to fix it to the tube at the proximal end. The device now being completely assembled, next is wrapped in paper save for the handle which projects beyond it.

It is sterilized, then, in the autoclave. These tubes may be prepared in quantity in the several sizes and used as desired.

With the proctoscope or sigmoidoscope in place, and if necessary held there with the aid of an assistant, the paper covering now is removed from the sterilized device. Next the distal cotton pledget is withdrawn, and the distal end of the device is passed quickly through a flame, and then the device is inserted through the instrument under the direct vision of the worker to a point directly over, but not touching the area from which the material is desired. Still under the vision of the worker, the applicator is pushed forward and the swab now is in contact with the sought-for involvement; then, with the glass tube in its original position with relation to the involvement and instrument, the applicator is drawn back into it and only then is the entire device withdrawn from the proctoscope or sigmoidoscope. Care should be taken that the distal portion of the glass container should touch neither the intestine nor any of the contents. As soon as possible thereafter, and immediately subsequent to the flaming of the proximal end of the device, the material-containing swab is removed through the proximal portion of the glass tube so as not to touch the distal end again. The swab is placed now either in a sterile test tube, or the appropriate media are directly inoculated.

By means of this glass tube which encloses the iron wire applicator and cotton swab, the working area through the instrument and within the bowel is much reduced, thus protecting the swab from coming into contact with any of the region surrounding the point from which material is secured, and also from being contaminated by contact with any part of the proctoscope or sigmoidoscope. The 17 cm. long tube is employed when the proctoscope is used; the 30 cm. long tubing, when the sigmoidoscope is used. The 8 cm. length, for securing material directly from the intestine through a fistula, will be discussed later.

2. The second procedure is one which permits the obtaining of contents, secretion or excretion for bacteriologic study, directly from the lower sigmoid through a sigmoidoscope, without contamination either by rectal contents or from other sources. It is a modification of the method described by Andrews and the author for the detection of protozoa from sigmoid contents. The method is as follows:

The patient is placed in Haynes's position, that is, the lower extremities remain on the table, while the trunk, flexed at the hips, is virtually at right angles to the lower extremities; the head rests on the forearms, which are flexed at the elbows and are supported by a footstool of convenient height. This position has been found preferable to the usual, well-known, knee-chest posture. In the Haynes's position the viscera are, by force of gravity, moved out of the pelvis, up toward and against the diaphragm. This reduces intrapelvic pressure and facilitates both instrumentation and the proper execution of this method.

Next, the anus is cleansed, first with green soap and water, then with alcohol if desired, and finally with a 1 to 1000 solution of bichloride of mercury. The right hand is now covered with a sterile glove, the first finger of which is inserted into the rectum so as to stretch the anal sphincter to render more easy the inserting of the sigmoidoscope, if for no other purpose. The sterile sigmoidoscope, covered with a sterile lubricant, next is inserted. The patient is ordered to take neither purge nor enema prior to this examination because we learned that any method which results in the thorough cleansing of the rectum will also result in a clean sigmoid with no gross material available at either point. If this is done he usually appears with feces in the rectum. In order for the feces to pass from the rectum into the sigmoid so as to secure material from the latter point without contamination from the former source, the subsequent steps are pursued:

With the instrument in the rectum near the feces encountered, the rectal content, diluted when necessary with sterile saline introduced into the sigmoidoscope through a sterile funnel placed in its proximal end, is drawn out through a sterile aspirator 35 cm. by 8 mm., by means of a suction apparatus. In this way the rectal feces are removed without affecting the material in the sigmoid. When the sigmoid is entered, the type applicator described above may be employed to secure through the sigmoidoscope the material desired without contamination from surrounding sources.

Distally lighted instruments are to be used in the execution of Methods 1 and 2. The proctoscope and sigmoidoscope used by the author each present a diameter of 16 mm., a diameter no greater than that of the average adult male index finger. An instrument, the diameter of which is as low as 12 to 13 mm., can be used in this work when necessary; however, the former size is to be preferred.

3. The securing of material for bacteriologic study directly from the intestine through a fistula is accomplished by using the iron wire applicator enclosed in a glass tube 8 cm. long. In this case, the paper wrapper having been removed, the sterilized device is inserted, not necessarily completely, directly into the fistula. Excepting that the subsequent steps cannot be performed in their entirety under the direct vision of the worker, they are, however, identical with those described for Method 1. The use of this type applicator in an intestinal fistula presents the advantage of not only preventing the swab from touching the protruding mucosa or adjacent skin, but also keeps the swab from coming into contact with the mucosa of the intestine beyond the fistulous opening upon entry and withdrawal. The contraindications to the use of this device are a fistulous opening too small to permit its entry into the intestinal lumen, and many appendicostomies in which both the opening and appendiceal lumen are too small.

STAIN: A New Method for Differential Staining of Plasma Cells and of Other Basophilic Cells, Hitchcock, C. H., and Ehrlich, W. Arch. Path. **9:** 626, 1930.

The staining fluid finally adopted consists of a mixture of a 2 per cent aqueous solution of malachite green with a 2 per cent aqueous solution of acridine red, in the proportion of one part of the former to three of the latter. This mixture keeps well and does not show any precipitation. A few weeks' ripening of the mixed solutions is of advantage, though excellent results are secured when the stain is employed as soon as prepared. Only Grubler's dyes have been used. Sections prepared by the paraffin method are passed through iodine in the usual manner. The iodine is removed by graded alcohols followed by thorough washing in running water for at least fifteen minutes; incomplete removal often results in precipitation of the stain. The slide is then flooded with the dye solution for from fifteen to thirty seconds.

The excess of solution is drained away and the slide is rapidly washed in water. It is then passed directly into absolute alcohol, where it is left only as long as the dye continues to be washed out in clouds. It is then transferred quickly into xylene. Occasionally, the brilliancy of the preparation is enhanced by restaining.

Comparative observations have shown that alcohol-fixed material is well stained by this method, but such sections are more deeply colored by the Unna-Pappenheim formula. On

the other hand, tissue fixed in corrosive sublimate or in Zenker-acetic acid solution yields better preparations when the malachite-green acridine-red mixture is employed. Results in fixation with Muller's solution plus formaldehyde have been unsatisfactory even when the slides have been subjected to preliminary overnight soaking in alcohol.

The cytoplasm of the characteristic cells of the Aschoff body is stained a rather dull, smudgy red, which varies considerably in depth from cell to cell, and is distinct in quality from the brighter hues seen in young or functionally active cells. In young subcutaneous rheumatic nodules may be found great numbers of basophilic cells, some with brilliantly staining protoplasm, others characterized by the dull dirty red of the Aschoff's body cell. Similarly staining cells are frequently encountered in other rheumatic tissues.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Esophageal Obstruction**

A COMPREHENSIVE volume on the physiology and pathology of the esophagus with outlines of therapy. Abundantly and beautifully illustrated. This work will be of especial interest to bronchoscopists and surgeons and to a lesser extent to roentgenologists and internists, whose interest will be primarily diagnostic.

Artificial Sunlight and Its Therapeutic Uses†

THIS volume is now in its fifth edition. Important additions since our previous review consist in more detailed discussions of disease conditions appropriate for actinotherapy, and the technic of treatment in the individual diseases, discussion of practical apparatus for determining the amount of ultraviolet light given out by any one machine, and discussion of the dangers and counterindications of treatment and the hazards of its improper administration.

Written, as it is, by one whose claim as an authority is justified, this volume should be well received by those who are interested in the subject.

Ker's Infectious Diseases‡

A COMPREHENSIVE textbook covering the following infections: measles, German measles, scarlet fever, smallpox, vaccinia, chickenpox, typhoid fever, diphtheria, erysipelas, whooping cough, mumps, and meningitis. This discussion, filling as it does, 594 pages, may be said to be very comprehensive.

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*Oxford Medical Publications. Esophageal Obstruction. Its Pathology, Diagnosis and Treatment. (Based on the Jacksonian Prize Essay of The Royal College of Surgeons of England for 1925. Published by Permission of the Council and Including a Hunterian Lecture Delivered at the Royal College of Surgeons in 1926.) By A. Lawerence Abel, M.S.(Lond.), F.R.C.S. (Eng.). Assistant Surgeon to the Cancer Hospital, London; Surgeon to the Kensington General Hospital; Assistant Surgeon to the Woolwich War Memorial Hospital; Surgeon to the London Lock Hospitals. Humphrey Milford. Cloth, pages 234. Oxford University Press. American Branch, New York. 1929.

†Oxford Medical Publications. Artificial Sunlight and Its Therapeutic Uses. By Francis Howard Humphris, M.D.(Brux.), F.R.C.P.(Edin.), M.R.C.S.(Eng.), L.R.C.P.(Lond.), L.M.(Rot., Dublin), D.M.R. & E.(Contab.). Fifth Edition. Humphrey Milford. Cloth, pages 340. Oxford University Press. American Branch. New York.

‡Oxford Medical Publications. Ker's Infectious Diseases. A Practical Textbook. Revised by Claude Rundle, O.B.E., M.D.(Lond.), M.R.C.S.(Eng.), L.R.C.P.(Lond.), D.P.H. Medical Superintendent, City Hospitals and Sanatorium, Fazakerly, Liverpool and Lecturer on Infectious Diseases to the University of Liverpool. Third Edition. Humphrey Milford. Cloth, pages 614. Oxford University Press. American Branch, New York.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Lipiodol in the Diagnosis of Thoracic Diseases**

AN ATLAS of chest roentgenograms, illustrating pathologic findings in which the intratracheal administration of lipiodol is of diagnostic value.

There are introductory chapters on the nature and value of lipiodol, technic of administration, and dangers. The volume will be of interest chiefly to roentgenologists and to internists whose major field of interest is the chest.

Bodily Changes in Pain, Hunger, Fear, and Rage†

THE theory of Cannon based on researches in his laboratory, that quite different types of emotion produce similar physiologic responses, is now a matter of general knowledge. It is a theory that has not gone without challenge, and in his second edition Dr. Cannon reproduces his experimental evidence, answers criticisms and produces additional evidence in substantiation of his theory.

It will be recalled that the dominant response to any strong emotion, according to the theory, is an outpouring of adrenin into the blood stream. Among the physiologic responses to this increased secretion are constriction of the visceral vessels and the vessels of the skin with dilatation of the blood supply of the muscles, thereby diverting more blood to the muscular system; a flooding of the blood stream with glucose which is promptly converted into energy in the muscles; an increase in the blood pressure and in the pulse rate facilitating carrying of oxygen and glucose through the muscles and the removal of metabolites; and hastening of the coagulation of the blood. Dr. Cannon shows in the present volume that adrenin experimentally lessens fatigue and that strong emotion increases the number of circulating red cells, and increases the respiratory rate. The value of all of these readjustments in a physical or emotional crisis appears obvious. Dr. Cannon localizes the seat of the emotion in the region of the optic thalamus.

There is an interesting chapter on the action of the emotions in producing organic disturbances such for example as hyperthyroidism.

It is not surprising that as a result of his physiologic and psychologic studies Dr. Cannon finds himself unable to support the contentions of the pacifists. Anger and rage have been dominant emotions since time immemorial and man is physiologically adjusted thereto. He does, however, in his closing chapter present an eloquent plea for alternative outlets to war.

Hemorrhoids‡

ARTHUR S. MORLEY has written a concise book on *Hemorrhoids.*

A detailed description of the author's treatment by the injection method, with selected case reports from a series of four thousand cases concludes the book, with the evidence in favor of the author's method of treatment.

The author injects at each sitting all the internal hemorrhoids present, or as many as possible. The author uses a solution of:

Acid Carbolic	gr. 48
Glycerin	2 ounces
Aquae distillatae	2 ounces

He believes that an average of four injections will cure most cases, and particularly warns against the injection of prolapsed hemorrhoids.

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*Oxford Medical Publications. Lipiodol in the Diagnosis of Thoracic Disease. By F. G. Chandler, M.A., M.D.(Contab.), F.R.C.P.(Lond.). Physician with Charge of Out-Patients, Charing Cross Hospital; Physician, City of London Hospital for Diseases of the Heart and Lungs, Victoria Park, E. and W. Burton Wood, M.A., M.D.(Contab.), M.R.C.P.(Lond.). Physician with Charge of Out-Patients, City of London Hospital for Diseases of the Heart and Lungs, Victoria Park. E. Humphrey Milford. Cloth, pages 133. Oxford University Press. 1928.

†Bodily Changes in Pain, Hunger, Fear, and Rage, an Account of Recent Researches Into the Function of Emotional Excitement. By Walter B. Cannon, M.D., S.D., LL.D., George Higginson Professor of Physiology in Harvard University. Second edition. Cloth, pages 404. New York and London, D. Appleton and Company, 1929.

‡Oxford Medical Publications. Hemorrhoids, Their Etiology, Prophylaxis, Treatment by Means of Injections. By Arthur S. Morley, F.R.C.S.(Eng.). Late Temporary Assistant Surgeon to St. Mark's Hospital for Cancer, Fistula and Other Diseases of the Rectum. Fourth Impression Revised and Enlarged. Humphrey Milford. Cloth, pages 122. Oxford University Press. American Branch. New York, 1929.

*Tweedy's Practical Obstetrics**

BETHEL SOLOMONS has rewritten an interesting edition of *Tweedy's Practical Obstetrics.* This edition contains 759 pages with 294 pictures and diagrams. The book is divided into eight parts:

1. Reproductive Organs and Embryology.
2. Normal Pregnancy, Labor, and the Puerperium.
3. Toxemias of Pregnancy.
4. Abnormal Labor.
5. Abnormal Puerperium.
6. Obstetric Operations.
7. The Infant.
8. Appendices. Including statistics, charts, and tests.

The book is easily read, and the various subjects are taken up in a clear, concise manner. The treatment of the complications of pregnancy is discussed, and usually the conservative treatment as generally advocated in this country is advised. Uterine and vaginal douches in obstetrics are mentioned frequently, but fortunately advised against in most cases. The use of the Voorhees bag is probably more often indicated in hospital practice than Solomons advises. Apparently he never uses the Voorhees bag. Pyelitis of pregnancy is not given any particular attention and the author does not mention cystoscopy for the stubborn cases. In this country we frequently give cystoscopic treatments. The prevention and treatment of shock in obstetrics are stressed, but the author does not advise transfusion as frequently as the authors in this country and no mention is made of the more common methods of transfusion now in use. The book will be especially useful to the general practitioner and student, as a practical guide.

Cancer of the Breast†

THIS is one of the series of Harper Medical Monographs designed to present in a clear yet succinct manner a thorough survey of a specific subject.

The importance of the subject covered in the present volume needs no emphasis. Dr. White has presented in a clear and authoritative way a very clear-cut survey of the present status of knowledge concerning malignant tumors of the breast, not only from the standpoint of pathology and diagnosis, but also the accepted methods of treatment. To complete the volume a section on histologic technic is included.

This little volume serves a useful and practical purpose and deserves a cordial reception.

The Use of the Microscope‡

IT IS quite within the bounds of probability that many of those who use the microscope frequently are more or less unfamiliar with many of the technical details and adjustments by means of which microscopic pictures may be seen at their best. To all such this present volume will be exceedingly useful, covering and explaining as it does in a clear and very readable way all of the uses to which microscopy may be applied.

Almost every chapter ends with an exceedingly useful summary of "practical points."

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*Oxford Medical Publications. Tweedy's Practical Obstetrics. Edited and largely written by Bethel Solomons, M.D., F.R.C.P.I., M.R.I.A. Master, Rotunda Hospital; Some time Gynecologist, Mercer's Hospital, Dublin; Consulting Gynecologist, Auteven Hospital, Kilkenny, Countess of Wicklow Hospital, Anklow and Newcastle Sanatorium; Vice President, Censor and Examiner, Royal College of Physicians, Ireland. Sixth Edition. Humphrey Milford. Cloth, pages 769. Oxford University Press. American Branch, New York.

†Cancer of the Breast. By W. C. White, M.D., Junior Surgeon, Roosevelt Hospital. Cloth, 221 pages, 17 figures. Harper & Bros., New York.

‡The Use of the Microscope. By John Belling, Cytologist, Carnegie Institute, Washington. Cloth, 315 pages, 28 illustrations. McGraw-Hill Book Co., New York.

There are also, in tabular form, lists of the various causes reducing the clarity and sharpness of microscopic images as well as the means whereby they may be avoided or rectified, and, at the end of the volume, a fairly comprehensive and useful glossary.

Fifty practical exercises and a list of one hundred questions assure a thorough understanding of the text.

The volume can be recommended to all who desire to secure maximum microscopic efficiency.

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*Statistical Methods for Research Workers**

THE fact that this book within five years has reached its third edition is prima facie evidence of its practical value to all those whose work involves the use of statistical data.

The purpose of the volume, well achieved, is to present in a relatively simple way methods for the statistical study of data. The book should be welcomed by all to whom such problems are presented.

Uterine Tumors†

THIS volume is a new addition to the series of Harper's Medical Monographs, a form of publication which is rapidly assuming an important position in the field of medical literature.

Uterine tumors are so frequently encountered by the practitioner and, if their potentialities are overlooked, constitute so dangerous a condition in the female, that the importance of a succinct survey of the subject requires no emphasis, nor need the fitness of Dr. Norris to present such a summary require comment.

The author wisely devotes most of the text to the diagnosis of uterine tumors, although treatment is touched in so far as it concerns other than purely surgical procedures.

The usefulness of the simple test for malignancy devised by the late John G. Clark is deservedly emphasized, as also is the problem confronting the pathologist asked to present dogmatic opinions from examination of curettage material.

The volume can be read with profit not only by the practitioner upon whom the responsibility for the recognition of uterine tumors first falls not infrequently, but also by those concerned with their treatment.

It can be well recommended as well serving the purpose for which it is intended.

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*Statistical Methods for Research Workers. By R. A. Fisher, Sc.D., Chief Statistician Rothamstead Experimental Station. Cloth, 283 pages, 111 tables. Oliver and Boyd, Edinburgh and London.

†Uterine Tumors. By C. C. Norris, M.D., Professor of Gynecology and Obstetrics, University of Pennsylvania. Cloth, 251 pages, 14 figures. Harper & Bros., New York.

The Journal of Laboratory and Clinical Medicine

VOL. XVI ST. LOUIS, MO., OCTOBER, 1930 No. 1

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Richmond, Va.

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Official Organ of the American Society of Clinical Pathologists

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Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

The Action of Insulin

RESEARCHES both in the laboratory and the clinic have established the fact that insulin given parenterally can adequately and harmlessly replace the internal secretion of this hormone by the pancreas. The evidence from the laboratory side has been derived by observing the behavior of completely depancreatized dogs injected twice daily with insulin during several years. After four years two such animals, when they ultimately died as a result of withholding the insulin for between four and five days, showed on *postmortem* examination no other condition than excessive fatness. The kidneys and liver were perfectly normal and no hardening of the arteries could be detected. Throughout the course of the insulin treatment, the animals were healthy in every way, active and friendly, free from infections and, in the case of one of them, capable of bearing pups without any complications. The carbohydrate tolerance of both animals was, as far as could be judged, just exactly the same at the time of their deaths as immediately following

the pancreatectomy four years previously. Hedon in Montpelier has recorded similar observations.

The evidence from the clinical side is equally convincing. Joslin, for example (Ann. Int. Med. **2**: 1001, 1929) states that when the total caloric intake is not excessive (and the diet is a mixed one), improvement in carbohydrate tolerance is to be expected as insulin treatment is continued with over a period of several years. He writes, "Diabetic mortality among individuals under twenty years of age in Massachusetts has almost reached the vanishing point. . . . . (and) the few who die are dying needlessly."

The only difference that can be observed in the laboratory and the clinical results is that the carbohydrate tolerance remains unchanged in the former, whereas it often increases in the latter, and this difference is easy to explain. In the totally depancreatized animal, no insulin is available except what is injected, whereas in the diabetic patient there must always remain some residue of pancreas which is not so completely destroyed by disease but that it can regenerate if given the chance to do so. This chance is afforded by giving the pancreas complete rest. To quote Joslin again, "Neither von Noorden with his 20,000 cases nor I with 7,000 believe we have encountered a complete diabetic"; that is, there must always be some endogenous insulin available and therefore some remnant of islet tissue must remain which, if given the chance, may serve as a focus from which new, healthy insulin-secreting cells may develop.

In view of these facts it seems to me inadvisable, if not indeed dangerous, to risk giving diabetic patients any of the supposed substitutes for insulin. Even the most powerful of these (synthalin), as judged from the power to induce hypoglycemia, falls far behind insulin as an antidiabetic agent and the supposed advantage of its being active when given by mouth is more than offset by the risk of damage to the liver which it entails. Among others, Mirlin has endeavored to devise some means by which insulin itself can be given by mouth, such as by mixing it with blood serum, but it cannot be said that his results are sufficiently convincing to warrant the risk of prolonged clinical trial.

How insulin acts remains as deep a mystery as ever, but one thing seems certain, viz., that its locus of action is primarily in the muscles. In some way it is necessary for the physiologic utilization of carbohydrate, and if we concede that other foodstuffs, both protein and fat, must pass through a carbohydrate stage before their ultimate utilization as fuel, then we must conclude that insulin is an essential hormone for the metabolism of all the proximate principles of food. In some way or other the action of insulin involves changes in the glycogen content of both the liver and the muscles. Injected without sugar into normal animals it causes the glycogen of the liver to decrease, but, as Cori and Cori have recently shown, if glucose be also injected in sufficient amount to maintain the blood sugar at its normal level, this depletion of liver glycogen does not occur. It is altogether likely, therefore, that the lowering of the blood sugar caused by insulin is responsible for the reduction in the glycogen content of the liver. But we must be careful not to conclude that hypoglycemia is the sole factor concerned in this

effect, for it is becoming more and more evident, as research proceeds, that the hepatic glycogen plays a much more important rôle in carbohydrate metabolism than is implied by calling it a storage material, in the same sense that starch is in the case of the potato. It is not a static but a dynamic substance. It represents the balance left over between two opposing processes which are constantly occurring in the liver cell, glycogen formation and glycogen breakdown, and when we remember that it is formed both out of preformed carbohydrate (glycogenesis) and from other substances (glyconeogenesis), we can see how a mere measurement of the amount remaining after death can tell us but little of what *antemortem* processes may have been occurring. Insulin may either increase or decrease the glycogen of the muscles. The greatest increase occurs when large amounts of glucose are added to the blood along with correspondingly large doses of insulin, and decrease when the hypoglycemia has been sufficiently pronounced as to cause convulsive symptoms. When the hypoglycemia is of insufficient degree to cause any convulsions or other symptoms, reduction of muscular glycogen still occurs and there is no evidence to justify the claim, which some have made, that increased muscular activity is alone responsible for it.

Even when the respiratory exchange is measured, it is often impossible to account for more than a relatively small proportion of the glucose which disappears. It will be evident in the light of these facts that it is very doubtful whether the balances which several investigators have attempted to draw between the amount of extra glucose actually disappearing from the organism and the increase in respiratory metabolism and glycogen deposition resulting from the injection of insulin can be regarded as at all accurate. One small error in any of the "items" of the accounts may make a great difference in the balance and such errors are easily made, even when the greatest care is taken in determining their values.

—*J. J. R. M.*

On Public Education

EVERY year the Congress on Medical Education and Licensure presents in the *Journal of The American Medical Association* a report and discussion of its activities. Although of distinct interest and definite importance to those engaged in the practice of medicine it is questionable, perhaps, if this matter arouses the general interest or receives the general attention it deserves.

The medical profession has long been concerned, and justly so, with the invasion of its field by unqualified individuals who vigorously disclaim the right of any agency to determine their qualifications or regulate their activities in the care of the sick "because, as they were not practicing medicine they should be exempt from the training of physicians." Nevertheless, it is common knowledge that all such individuals *are* engaged in the practice of medicine through the very obvious device of entering its domain by the back door of irregular, pseudoscientific instruction in the idiosyncrasies and peculiarities of cults and fads.

The responsibility for this situation, the existence of which cannot be gainsaid, rests, of course, in the credulity of the public arising from lay ignorance in matters pertaining to health and disease.

Efforts to remedy this lack of understanding by broadcasts and other means have met with varying success. This is understandable, for the healthy individual seldom spends much time in the consideration of possible illness and, unless the speaker has the human interest of Amos 'n' Andy, or the combination of interest and volubility possessed by Floyd Gibbons, the listener more often "tunes out" on the medical program and "in" on the dance orchestra than vice versa.

Moreover, until some means can be found to regulate what goes out on the air, there is nothing to prevent the quack, the cultist, and the vicious type of faker from broadcasting what he will as often as he will. Indeed, this is now being done from more than one privately owned station, not to mention the more or less absurd bits of medical misinformation to be garnered between dances on the program of some of the more reputable stations.

All of which makes it more obvious that the proper and most effective time, place, and opportunity for lay education in these matters is the doctor's office. Here the patient is eager and anxious to know all that he can of the workings of his bodily mechanism and what has thrown it out of gear. His interest is keen and focused on the matter at hand and all that is told him then falls upon an attentive and receptive mind. Properly informed he then becomes a source of information to others. Passed on with a handful of pills and a snap diagnosis, his last state is worse than his first, and it is not strange that he is later impressed by the cultist who not only presents a voluble statement as to what's what but backs it up with an x-ray of the spine in the bargain.

The responsibility for the education of the public rests squarely upon the shoulders of the practicing physician nor can he evade likewise some, at least, of the responsibility for the receptiveness of the lay mind in general to misinformation.

Public education by the physician need not necessarily be concerned with highly abstract and abstruse subjects. Long dissertations upon the varying theories as to the cause of cancer or the mechanism concerned in the production of myasthenia gravis are not nearly so important nor so acutely necessary as far more simple matters.

For example, the term "Doctor." The physician whose sign reads "DR." makes no distinction, in the public mind, between himself and the various "Doctors" of all kinds who abound in the land. The same is true of the physician who fails consistently to label himself, speak of himself and sign himself "M.D."

A simple thing, and a small thing, perhaps, but life is made of trifles and existence is, after all, a matter of coordinated minutiae.

The physician who will not take the time or spend the effort to inform his patients has no one but himself to blame if their ignorance grows apace and leads them into irregular paths.

Misinformation can be passively as well as actively conveyed. The physician who sends his patient to "Dr." so and so for some laboratory procedure when the so-called "Dr." is simply a technician who has opened a laboratory, is simply detracting from the value and dignity of the title he himself worked long and hard to obtain, and the man whose therapeutics consists mainly in the prescribing and dispensing of proprietary formulae is, in effect, placing himself on the level of the drug clerk practicing over the counter.

Surely, too, those various thousands who have sold their birthright, as it were, for a mess of pottage in the shape of a carton of cigarettes, a handful of yeast cakes, or a package of soap and the like are not entitled to wonder why the title "Doctor" is rather promiscuous these days.

The profession of medicine has a heritage of dignity which such things do little to uphold, and it is well to remember that the public at large draws its conclusions, not from prolonged study of the history of medicine nor from the lives of its outstanding heroes, but from the individual practitioner with whom, as individuals, it comes into contact.

—*R. A. K.*

Erratum

In the article by Vilkomerson, New Rapid Paraffin Method for Tissue Sections, December, 1929, page 291, second paragraph from top, line 3, after the word "slide," it should read: "provided it is placed on the asbestos plate, just above the electric coils, when the paraffin just begins to melt."